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The First Record of *Escherichia coli* O157: H7 Isolated from Wastes and Associated Houseflies in Egypt

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Abstract: Fly population density were estimated in relation to five different types of wastes, household, slaughterhouse, agricultural, hospital and general waste types in our habitats.The highest fly density was estimated from the household type as 370±199.9 fly/trap. Waste contents were sorted including urban, suburban and randomized areas in our country, *Escherichia coli* O157:H7 was isolated for the first time from the five types of wastes and their associated flies. From the screened eighteen localities, five only proved to be positive for *E. coli* O157:H7 from both waste samples and their associated flies.The Molecular studies including PCR method for amplification of a 16S rRNA gene region of E. coli and that for detection of Shiga like toxins *stx*1, *stx*2 and *eae* genes proved to be positive within our isolates.

Key words: Housefly-Vector · Vectorial Capacity · Breeding Places · *E. coli* O157:H7 Outbreak

INTRODUCTION

Our environmental conditions and the slow treatment of garbage increase fly breeding places and subsequently the circulation of disease causative organisms' especially enteric bacteria which are transmitted by flies [1-3].

Diarrheal diseases are considered the most important cause of childhood mortality worldwide [4]. *Escherichia coli (E. coli.), Campylobacter* spp., *Salmonella and Shigella* spp. are the most virulent causative organisms of such diseases.

E. coli O157:H7 that produces Shiga like toxins (STEC) becomes life threatening after its responsibility of the largest outbreak in USA in 1993, [4] and the following outbreaks in European and Asian Countries [5-9]. Since that time this bacterium is becoming one of the most important pathogens. An estimation of 73480 cases of illnesses, 62458 hospitalization and 61 deaths occur each year referred to this pathogen in USA only [10]. The clinical manifestations after infection by this bacterium include hemorrhagic colitis, hemolytic uremic syndrome, abdominal cramps, fever and kidney failure [11].

Pathogenic isolates of *E. coli* O157:H7 processes a number of virulence factors that are important for the ability to cause disease. This virulence factors include as the most important and affecting adhesion molecules which encoded by *aea* gene and the Shiga-like toxins (stx1, stx2) [12].

The aim of the present study was to investigate the presence this hemorrhagic bacterium, in flies and wastes of its breeding places in Egypt and correlate between its prevalence and type of wastes in different localities.

MATERIAL and METHODS

Sampling Sites: Eighteen sampling sites, representing nine localities within Cairo governorate were visited twice to collect and estimate fly density breeding on wastes collected from these sites during two successive years. The chosen areas, (Table 1), representing high, moderate and low socioeconomic level habitats including El-Demerdash hospital area (sites 1, 2), El-Deweka, (sites 3, 4, 5), Cairo-Ismailia road (sites 6, 7), Monshaat Nasser, (sites 8, 9), El Pasateen district (sites 10, 11), Ain Shams district (sites 12, 13), El-Obour Market (sites 14, 15), Heliopolis area (sites 16, 17), El Marg area (sites 18) were visited during our study.

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Table 1: Locations and sites visited during the study for waste and fly collections within Cairo governorate.

Governorate	Location	Site	
Cairo governorate	El-Demerdash hospital	Hospital main garbage area.	(site1)
		Food processing area.	(site2)
	El-Deweka	Garbage area.	(site3)
		Herbarous area.	(site4)
		El-Souk area.	(site5)
	Cairo Ismailia road	Farm (1) at Ahmad Orabi	(site6)
		Farm(2) at Ahmad Orabi	(site7)
	Manshaat Nasser	Sorting household wastes) area	(site8)
		Area between houses	(site 9)
	El-Pasateen distrit	Main slaughterhouse of Cairo	(site10)
		Souk EL-Gomaa	(site 11)
	Ain Shams district	Cafeteria	(site12)
		Colleting area of garbage	(site13)
	El-Obour Market	Fish store	(site14)
		Fruit market	(site15)
	Heliopolis area	Restaurant area (Tivoli)	(site16)
		Souk Haroon	(site17)
	El Marg area	Farm area	(site18)

Field Collection of Fly and Environmental Samples: Flies were collected using sticky traps as expired X-ray films, covered with a layer of sticky glue with yeast extract and sugar and hanged in each site for 24 h and collected for fly density estimation. Different waste samples within each site were collected in sterile zip bags for sorting and bacterial isolation. Sterile areal nets were used to collect fly samples used in bacterial isolation.

***Isolation of *E. Coli* O157: H7 from Samples:** Fly samples were homogenized in 0.5 mL of phosphate-buffered saline and centrifuged for 3 min at 3000 rpm, while environmental sample was immersed in sterile peptone water (0.2%) or bacteriological peptone 0.1% with shaking, then both samples were used to inoculate sorbitol-MacConckey agar plates as specific media for *E. coli* O157:H7 isolation [13]. Growing non-sorbitol fermenting colonies were described and photographed after determination of cell morphological and staining properties. Isolated colonies were used for serotyping and PCR tests.

Serotyping of *E. Coli* O157 by Latex Agglutination Kit: Non sorbitol fermenting colonies from Sorbitol MacConckey agar, (SMA), were subjected to agglutination slide with the *E. coli* O157 Latex kit (Oxiod, DR 620 M) and the agglutination colonies were further processed for definite confirmation according to the procedure of latex kit (Oxoid, Basingstoke and Hampshire, England) [38].

DNA Extraction and PCR Amplification: The isolated bacteria was subcultured on tryptone soy broth and incubated overnight at 37°C for DNA extraction according to manufacturer's instructions of the used kit (QIAGEN Lot No. 136243308).PCR reactions were performed with Thermal cycler (Biometra, T professional). Amplification of a 16S rRNA gene region from *E. coli* was performed. The method was capable of discriminating *E. coli* from other enteric bacteria, including its closest relative, *Shigella*. PCR reactions were performed according to Sabat [14].

The sequence of 16s rRNA (forward, targeting bases 75 to 99; 5'-GGAAGAAGCTTGCTTCTTTGCTGAC-3') -(reverse, targeting bases 594 to 619; 5'AGCCCGGGGATTTCACATCTGACTTA3').

The resultant amplicon was 544 bp and then the presence of the Shiga toxins was assessed in all strains using the *stx1* primer:

(Stx1 F5'CAGTTAATGTGGTGGCGAAGG3),
(Stx1 R5' CACCAGACAATGTAACCGCTG 3'),

The resultant product was 348 bp and *stx2* primers with its sequence as follow:

(Stx2 F5' CCATGACAACGGACAGCAGTT-3').
(Stx2 R5'- CCTGTCAACTGAGCACTTPTCGR-3').

The resultant product was 590 bp as described by Feng and Monday [15]. The presence of the intimin determinant was assessed in all strains by PCR using the *eae* primers, (aea F5' GTGGCGAATACTGGCGAG3'), (aeaR5'CCCCATTCTTTTTCACCG-3') the resultant product was 860 bp as described by Gannon *et al.* [16]. A well-characterized STEC O157:H7 strain was used as the positive control (ATCC 43895).The electrophoretic products were visualized by UV transilluminator (Biometra) and photographed by Gel Documentation System including BioDocAnalyze (BDA) Software (Biometra) for measuring and analyzing the PCR results.

Statistical Analysis: The data were given as individual values and as means (X) ± standard deviation (SD). Comparisons between calculated means were analyzed using least significant difference (LSD) test. Differences were considered significant at $P < 0.05$. All statistical analyses were performed using the statistical software SPSS, version 15.

RESULTS

Description and Characterization of Collecting Waste Types: Wastes collected from eighteen different sites, representing localities of different socioeconomic levels and sanitary conditions, in our country, were classified according to waste contents to five types; household, slaughterhouse, agricultural, hospital and general waste type. The description of these types is involved in (Tables 2, 3, 4, 5 and 6).

Impact of waste type on fly density and prevalence of *E. coli* O157:H7: Results in tables (2, 3, 4, 5 and 6) include the estimated fly density collected from visited sites and the percentage occurrence of *E. coli* O157:H7 from both houseflies and collected waste samples.

Wastes of household type, representing in Table (2) was proved to be the favorite for flies to breed. The highest fly density was recorded within site (13) at Ain Shams district, (370 ± 199.91 fly/trap). It also includes the lowest value of fly collection (4.00 ± 3.35) at site (2) within El Demerdash hospital.

Table 2: Impact of household wastes on housefly density and prevalence of *E. coli* O157:H7

				Prevalence of *E. coli* O157:H7			
				Fly		Environmental waste	
Site	Constituent of wastes	Total fly no	Mean ± SD	n	%	n	%
Site (13): Ain Shams district,	Animal bones food remainder, papers and Plastics	2220	370 ± 199.91	3(30)	6	0 (10)	0.0
Site (8): Sorting household waste area (Manshat Nasser)	Animal manure-food remainder plastics and papers and baby pampers and fermented matters	2116	352.62 ± 29.48	6(101)	5.9	0 (10)	0
Site (9):Area between houses (Manshat Nasser)	Papers, food remainder, bird manure and dead animals	1629	217 ± 81.06	0 (50)	0.00	1 (12)	8.33
Site (3):Garbage area. El-Deweka	Shaved hair, fermented food matters, animal and poultry manure and remainder	1574	262.33± 183.01	5(50)	10	3(20)	15
Site (2):Food processing area in El-Demerdash hospital	Rotten fruits and vegetables, meat and poultry remainder	24	4.00 ± 3.35	1 (20)	5	0 (5)	0.00

Table 3: Impact of slaughterhouse wastes on housefly density and prevalence of *E. coli* O157:H7

				Prevalence of *E. coli* O157:H7			
				Fly		Environmental waste	
Site	Constituent of wastes	Total fly no	Mean ± SD	n	%	n	%
Site (14): fish store El Obour market	Fish viscera, Shrimp remainder	93	15.50 ± 3.51	0 (13)	0.00	0 (5)	0.00
Site (10): The main slaughter house of Cairo, El Basateen area,	Animal vesiraand manur, sheep and Baffallo remainders	1237	206 ± 29.12	2 (30)	6.66	2 (10)	20
Site (17) :Souk Haroon Heliopolis area	Meat remainder, skin, feather poultry vesira animal,stuffs	35	5.82 ± 2.78	0 (11)	0.00	1(10)	10
Site (5) : El-Souk area El-Deweka	Animal skin, bones, loodandmanur	1627	227.17 ± 81.06	3 (28)	10.7	1 (6)	16.6

Table 4: Impact of agricultural wastes on housefly density and prevalence of *E. coli* O157:H7

				Prevalence of *E. coli* O157:H7			
				Fly		Environmental waste	
Site	Constituent of wastes	Total fly no	Mean ± SD	n	%	n	%
Site (4): Herbarous area, El-Deweka	Decayed plant leaves heaps of dung.	374	62.33 ± 6.86	2 (43)	4.6	0 (3)	0.00
Site (15): Fruit Market, El- Obour area	Rotten fruits	117	19.50 ± 2.81	1 (20)	5	0 (5)	0.00
Site (6): Ahmed Orabi area, Farm(1) Cairo Esmalia road	Grass, dung, straw, mud and plant leaves	144	24.00 ± 10.47	4 (50)	8	1 (6)	16.66
Site (18) : Farm area, El-Marg area	Cows and sheep dung, plant leaves	1048	174.67± 63.30	6 (40)	15	2 (9)	22.22
Site (11): Souk EL-Gomaa, El pasateen	Rotten vegetables and fruits	797	132.83± 33.18	0 (10)	0	0 (5)	0.00

Table 5: Impact of hospital wastes on housefly density and prevalence of *E. coli* O157:H7

				Prevalence of *E. coli* O157:H7			
				Fly		Environmental waste	
Site	Constituent of wastes	Total fly no	Mean ± SD	n	%	n	%
Site (1): Hospital disposal garbage area El Demerdush hospital,	Papers, food wastes, gauze, bloody cotton and strings	797	132.83 ± 33.10	0 (25)	0.00	0 (10)	0.00

Table 6: Impact of general wastes on housefly density and prevalence of *E. coli* O157:H7

				Prevalence of *E. coli* O157:H7			
				Fly		Environmental waste	
Site	Constituent of wastes	Total fly no	Mean ± SD	n	%	n	%
Site (7) :Ahmed Orabi district- Farm 2	Food remainder, mud, rotten leaves and animal manur	851	141 ± 54.8	2 (31)	6.5	0 (5)	0.00
Site (12): Cafeteria, AinShams	Cafeteria wastes	144	24.00 ± 10.47	0 (10)	0.0	0 (5)	0.00
Site (16): Restaurant area Heliopolis area	Burger meat, bread, tea bags plastic cups, vegetable remainders, burger meat.	81	13.50 ± 6.72	0 (9)	0.0	1(10)	10

The highest fly densities associated with slaughterhouse wastes, (Table 3), were from sites (5, 10) representing El-Deweka area with fly density (227.17 ± 81.06) fly/trap and El-Basateen area (206± 29.12) fly/trap. The lowest fly density associated with slaughterhouse type was in El-Obour market, site (14) with mean fly density (15.50 ± 3.51).

For agricultural type of waste, (Table 4), the highest fly density was recorded at sites (18, 11) representing El-Marg and El-Pasateen areas, with fly densities (174.67±63.30 and 132.83 ± 33.18) fly/trap. While site (15) at El-Obour market recorded the lowest density as (19.50±2.81) fly/trap. Collection from hospital wastes recorded moderate fly density with mean 132.83 ±33.10 fly / trap (table 5) site (1). For general waste type, Table (6), a relatively low fly collected density ranged from (141± 54.8 to 13.50± 6.72) fly/trap.

Isolation of *E. Coli* O157: H from Samples: All suspected bacterial isolates on SMA, during this study are proved to be *E. coli* and has the amplicon (544 bp product) using the primers 16SrRNA which represents the conservative gene region in *E. coli* and specific to characterize *E. coli* strains (Fig.1). *E. coli* O157:H7 was isolated from flies of all sites except sites (1, 9, 11, 12, 14, 16 and 17) where flies gave negative results for isolation of this bacterial strain. The highest isolation prevalence from flies was recorded from a farm in El Marg area with 15%, (Table 4) site (18),followed by El Deweka (Table 2) site (3). The lowest percentage of occurrence was recorded from flies collected from El Deweka- site (4) as-(4.65%) as shown in (Table 4).

All waste types were positive for isolation except the hospital type, (Table 7). Only five sites (3, 5, 6, 10 and 18) are sharing isolates of *E.coli* O157:H7 from both wastes and associated flies, representing household,

Table 7: Prevalence of *E. coli* O157:H7 in relation to type of waste and fly density

		Prevalence of *E. coli* O157:H7			
		Fly		Environmental waste	
Type of waste	Total fly no	n	%	n	%
Household	7563	15 (251)	5.9	4(57)	7.01
Slaughterhouse	2992	5(82)	6.09	4(41)	9.75
Agricultural type	2480	13(163)	15.85	3(28)	10.71
Hospital type	797	0(25)	0	0(10)	0
General wastes	1076	2(50)	4	1(20)	5

Table 8: Percentage occurrence of *E. coli* O157:H7 from sites sharing isolation from flies and wastes:

		prevalence of *E. coli* O157:H7			
		Fly		Environmental waste	
Type of waste	site	n	%	n	%
Site (3):Garbage area of El-Deweka	Household	5 (50)	10	3(20)	15
Site (5): El-Souk area of El-Deweka	Slaughterhouse	2(28)	10.7	1(6)	16.66
Site(6): Ahmed Orabi area, (Farm 1)	Agricultural	4(50)	8	1(6)	16.66
Site(10): Main Slaugh-terhouse	slaughterhouse	2(30)	6.666	2(10)	20
Site (18): EL-Marg area	Agricultural	6(40)	15	2(9)	22.22

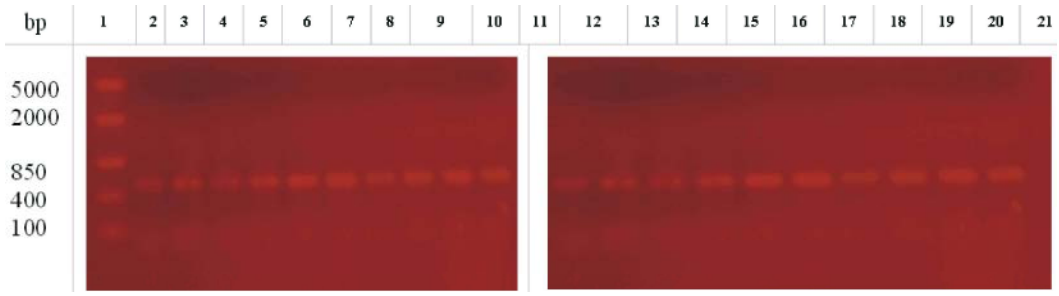

Fig. 1: 16S rRNA amplified region (544 bp) from isolated *E. coli*.

1-100 bp DNA ladder	2-standard*E.coli* O157:H7	3-Fly - site 13
4-Fly - site 8	5-Fly - site 3	6-waste - site 3
7- Fly - site 7	8-Fly - site 10	9-waste - site 10
10-waste - site 17	11-Fly - site 4	12-Fly - site 5
13-waste - site 5	14-Fly - site 15	15-Fly - site 6
16-waste - site 6	17-Fly - site 18	18-waste - site 18
19-Fly - site 2	20-waste - site 9	21-Waste-site 16

slaughterhouse and agricultural type of wastes, (Table 8). The agricultural wastes, (Table 4) site (18) recorded the highest percentage of occurrence of *E. coli* O157:H7 as 22.22%, from waste samples. Although the highest collected fly samples (7563 flies) was recorded from household type of wastes (table 7), the percentage of bacterial prevalence was not high recording 5.9% from flies and 7.10%from waste samples. Some sites were negative for waste and isolation of *E. coli* O157:H7, as sites (1, 2, 4, 7, 8, 11, 12, 13, 14 and15).

Serotyping of E. *coli* O157:H7: Results in using the latex agglutination kit, proved that all bacterial isolates (eight from environmental samples and eleven from flies) were all positive for *E. coli* O157:H7.

Fig. 2: Amplicons generated by amplification of *stx*1 gene of *E. coli* O157:H7 (348 bp)

1-100 bp DNA ladder	5-Fly - site 10	9- waste - site 5
2-standard *E. coli* O157:H7	6-waste - site 10	10-waste - site 18
3-Fly - site 3	7- Fly - site 6	11-Fly - site 5
4-waste - site 3	8-waste - site 6	12-waste - site 18

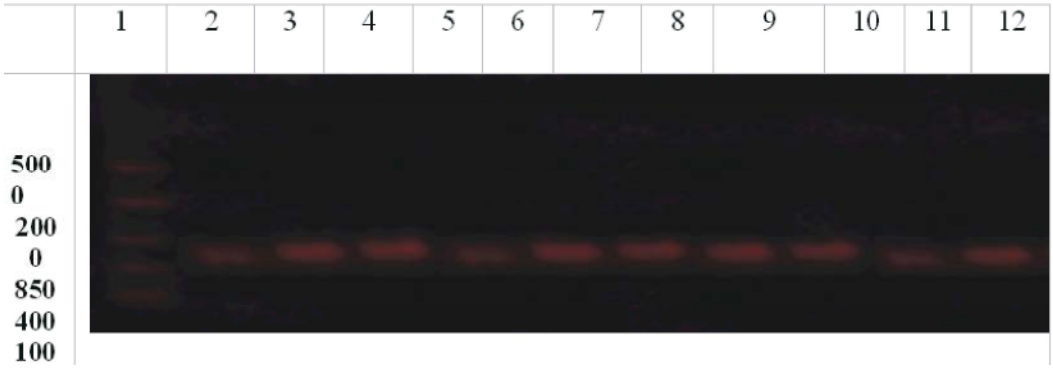

Fig. 3: Amplicons generated by amplification of *stx*2 gene of *E. coli* O157:H7 (590 bp).

1-100 bp DNA ladder	5-Fly - site 10	9-Fly - site 18
2-standard *E. coli* O157:H7	6-waste - site 10	10-waste - site18
3-Fly - site 3	7- Fly - site 6	11-Fly - site 5
4-waste - site 3	8-waste - site 6	12-waste - site 5

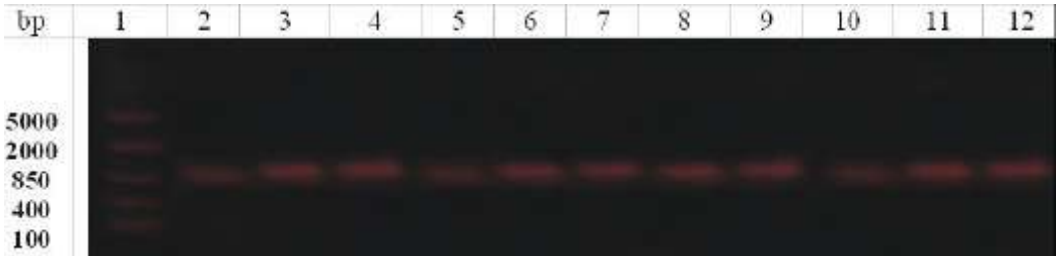

Fig. 4: Amplicons generated by amplification of eae gene of *E. coli* O157:H7 using eae primer.(860 bp)

1-100 bp DNA ladder	5-Fly - site 10	9- Fly - site 18
2-standard *E. coli* O157:H7	6-waste - site 10	10-waste - site 18
3-Fly - site 3	7- Fly - site 6	11-Fly - site 5
4-waste - site 3	8-waste - site 6	12-waste - site 5

Detection of *stx*1 and *stx*2 with PCR: Detection of toxic genes was carried out for isolates sharing presence of O157:7 from flies and wastes of the same sites (3, 5, 6, 10 and 18), (Table 8).

Amplicons of 348 bp for the amplification of *stx*1 gene within the isolates of *E. coli* (STEC) from flies and wastes of the mentioned sites proved positive results, (Fig2). In (Fig.2), Lane (1) representing DNA ladder as marker and lane(2) represents standard strain of *E. coli* O157:H7. Negative results for stx1 detected for isolates at sites (10, 12) representing the main slaughterhouse of Egypt- El-Pasateen area and wastes of a cafeteria from Ain-Shams district. PCR amplicons of *stx2* gene represented in (Fig.3) as a band size of 590 bp. All tested

sites (3, 5, 6, 10 and 18) showed positive bands proving the presence of *stx*2 gene. Amplification of *eae* gene using *eae* primer showed in (Fig. 4) as amplicon of 860 bp size, indicating the presence of this gene in common five samples of flies and wastes, the reaction was positive for all tested sites.

DISCUSSION

Houseflies, *Musca domestica* (Diptera : Muscidae) have long been considered vectors or transporter of pathogenic microorganisms [17].In our survey, sites of Manshaat Nasser (Table 2) site (8) and Ain Shams district (Table 2) site (13) recorded the highest fly density during this study (352.62±29.48 and 370±199.91)fly/trap, respectively. Manshaat Nasser and Ain Shams areas are of high population density and wastes are left for many days leading to fermentation of organic components which create favorite sites for fly breeding habitats. Sites (8 and13) representing household type of wastes containing food reminders, corps of dead animals and dippers with feces.

Flies of these sites are positive to *E. coli* O157:H7 recording relatively moderate percentage of prevalence, (5.9% and 6.0%) respectively while isolation from wastes recording 0% for site (13) and site (8). We cannot isolate *E. coli* O157:H7 from that site which recording high fly density with a mean value (352.62 ± 29.48) fly/trap. The estimated fly density associated with slaughterhouse wastes was also high recording (206± 29.2) fly/trap, (Table 3) site (10).This site represents the oldest governmental slaughterhouse in Cairo, surrounded by high residential population density and accordingly the amount of wastes. Isolation percentage of *E. coli* O157:H7 from flies of site (10) was recorded 6.66 %, while isolation from waste samples recording 20%. High percentage of isolation puts the slaughterhouse wastes and associated flies as important source of possible infection. Animal manure, viscera and meat reminders are the main reservoir for such hemorrhagic bacteria. The local shedding of zoonotic bacteria in slaughter animals was correlated with the hazard of carcass contamination with pathogenic bacteria from wastes or flies in open trucks with uncontrolled temperature [18]. This will increase the risk for infection; at low storage temperature of meat products the hemorrhagic E *coli* remains viable with the ability to produce shiga like toxins. Vazirianzader [19] could isolate these bacteria from slaughterhouses.

Slaughterhouse wastes in site (17) (Table 3) representing meat market within Heliopolis area, although fly density was low (78.17 ± 42.19 fly / trap) and negative for *E. coli* O157:H7 the bacteria was isolated with prevalence reached 10 % from wastes of that site which include meat reminders, bones and feces of animals. Besser [20] proved that feces, contaminated food and water are sources of *E. coli* O157:H7 contamination. Heliopolis area is well urbanized area where good sanitation measures preventing the breeding places of flies. Contamination of wastes may be occurred in slaughterhouses before reaching butcher shops. Estimated fly density accompanied with agriculture wastes, (Table 4), showed that the highest fly density was recorded from site (18), in El-Marg area which is highly randomized area, no sanitation measures is followed, petting zoo are beside people houses. It is an agricultural area and residents built their houses within the fields and rear animals at the same places.

Fly density recording (174.67 ± 63.30) fly/trap, (Table 4), at site(18), isolation of *E. coli* (STEC) recorded 15 % prevalence, while isolation from wastes recorded the highest percentage of occurrence of this hemorrhagic bacteria reaching 22.22 %. Animal feces and fertilizers play a role in contaminating vegetables [8]. Cattle with their manure are considered the main reservoirs of this pathogen [21, 22].Pathogen isolation from flies and its breeding places as agricultural wastes was recorded also at a farm along the Cairo-Ismailia road (Table 4) site (6), although fly density was relatively low (24.00± 10.47) fly/trap. We could isolate STEC from flies (8%) and wastes (16.66 %).

It appears that fly density is not the most important factor in dissemination of STEC but the type of waste it breed.

The quick treatment of hospital wastes (Table 5) site (1) appeared to make it unfavorable place for flies to breed and for bacteria to circulate. Isolation from wastes and flies was always negative, although Rady [23], isolated pathogenic bacteria from hospital wastes.Studying on wastes of general type (Table 5) which represents more than one type of wastes, the fly densities including in sites (7,12 and 16) were relatively low except collection from Ahmed Orabi farm (site 7) with fly density 141.00 ± 54.8 fly/trap.

Isolation of STEC from this site was positive for flies and negative for its wastes,it was also realized that site (12) representing cafeteria wastes was negative for isolation from flies or environmental samples, waste component of that site did not contain food remainder, meat or vegetables as in case of household or agricultural wastes, although this cafeteria was included in area of

moderate sanitation conditions, Ain shams area, with leakage of sewage and high rate of waste disposing. Restaurant area of Heliopolis (Table 6) site (16) is a clean area, peoples of such locality are of high socioeconomic level and sanitation facilities are considered. A low fly density for restaurant area -Tevoly- was recorded as (13.5 ± 6.72 fly / trap) with negative isolation of STEC from flies and 10 % from waste samples this is due to environmental sample composition which include burger, ground meat and vegetable remainder which considered another source of STEC contamination as mentioned [4, 6, 24, 25 and 40], while others [8, 26] correlate between increasing of *E. coli* O157:H7 prevalence in cattle and high fly population during summer months.

This study provides the first documented evidence that housefly carry the hemorrhagic bacteria *E. coli* O157:H7 in Egyptian localities. Incriminating flies to carry *E. coli* O157:H7 was suggested [1, 4, 7, 9, 27]. We can assume that the agricultural and slaughter waste types which include rotten vegetables, animal parts and excreta, are of high importance in dissemination and circulation of *E. coli* O157:H7 recording 15.85% and 6.09 % from flies and 10.71% and 9.75% from waste samples respectively, as prevalence percentage in our habitat, (Table 8).

The set of PCR primers used to target 16S rRNA which identify sequences conserved with *E. coli* is very important test. Results in (Fig.1) proved that all bacterial isolates were *E. coli*. Using DNA technology minimize false negative results [14].

The shiga like toxins producing element is the most important characteristic of *E. coli* O157:H7, shiga toxin (stx1) and shiga toxin II (stx2). Bloody diarrhea and abdominal cramps result from *E. coli* O157:H7 infections and are often initiated when endothelial cells in the digestive tract or kidneys are destroyed by these toxins [20].The severity of STEC infections correlate to the amount of bacterium ingested and amount of shiga toxins produced. Unfortunately, low quantities of each these bacteria can initiate infection in human [4]. Shiga toxin is not only the virulent factor in STEC, intimin is a major concern as this is the adhesion molecule responsible for attachment of STEC organism to the epithelial cells of intestine which can cause structural modifications and lesions, *eae* gene is responsible for encoding this adhesion molecule [4, 28, 29].

Our results for detecting *stx*1 (Fig. 2) proved that all fly and waste isolates carry *stx*1 gene except isolates from site (18) representing El Marg area with 22.2 % bacterial prevalence from wastes and 10.71% from associated flies of the same site. Amplicons of *stx*2, (Fig.3) proved that all isolates from flies or wastes of all sites including site (18) were positive for detection of the gene of this toxin. Absence of stx1 from some isolates means the presence of more than one strain of *E. coli* O157:H7in our habitat. While the presence of stx2 in isolates from waste samples and associated flies and absence of *stx*1 from isolates of waste sample and flies of the same site (18), incriminate houseflies to carry this pathogen from visiting wastes.

For detecting *eae* gene (Fig. 4) all isolates proved to carry this gene.Shiga toxin producing *E. coli* (STEC) proved to produce either stx1 or stx2 or both toxins, this phenomenon is recorded previously by Yokoyama *et al*. [30] and Yokoyama *et al*. [31]. Severe human disease thought to be linked to stx2 more than stx1 which appear to be more homogenous with only one variant than stx1 with five variants, [32]. *Stx1* is usually associated with a symptomatic or mild disease [33]. It was reported that the combination of *eae* and stx2 genes has been more frequently isolated from *E. coli* serotypes associated with human diseases [34]. Also, it was found that *stx2* was more prevalent than *stx* 1 during isolation of STEC from animal feces [32, 35, 39] reported that *stx2* gene was dominating than *stx1* in *E coli* pathogenic strains, it was found that only 50% of twenty four STEC isolates collected during research were carrying *stx1*[36]. On the contrary [37, 40] stated that the majority (60%) of studied STEC strains by his team carried *stx*2.

Mitigation: *E. coli* O157:H7 has become one of most significant food borne pathogens that has gained increased attention in the recent years. So, certain recommendations should be followed as a safe guard: improvement of clinical laboratory methodology for diagnosis, implementation of hygienic measures in farm animals and epidemiological surveillance for *E. coli* O157:H7 based on advanced technology.

- Wastes especially, household, slaughter and agricultural types are important sources of *E. coli* O157:H7 wastes need quick treatment by governmental authorities in our country.
- Fly densities are correlated to waste type. Sorting of wastes should begin from houses by correct method using tightly closed plastic bags of different colors provided from municipal authorities and collected from houses daily, also restrictions must be applied on shops which throw wastes in roads.
- Regulations for such aspects concerning getting rid of wastes should be promulgated by authorized agencies.

- We should have inspection programs about food processing.
- Ground meat should be cooked at high temperature in order to ensure killing of the shiga toxin producing *E. coli*.
- Agriculture wastes including rotten vegetables and fruits seem to carry the pathogen, contaminated vegetable when used in salad without washing or peeling can be cause of food borne illnesses. There is a need for vigorous washing of vegetables with safe running tap water before consuming to reduce bacteria clinical dose.
- There is direct need for care in abattoir waste disposal to avoid direct contact and also prevention of cross contamination to adjacent land or area.
- Proper washing of hands to those who working in agricultural affair, slaughter and food processing is a must.

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