Al-Azhar Bulletin of Science

Volume 34 | Issue 3 Article 5

2023

Section: Botany and Microbiology; Zoology

Forensic flies as carries of pathogenic bacteria associated with a pig carcass in Egypt

Ahmed H. Zaher
Zoology and Entomology Department, Faculty of science, Al- Azhar University, Nasr City, Cairo-11884, Egypt

Mohammed M. Kabadaia
Zoology and Entomology Department, Faculty of science, Al- Azhar University, Nasr City, Cairo-11884, Egypt

Kotb M. Hammad
Zoology and Entomology Department, Faculty of science, Al- Azhar University, Nasr City, Cairo-11884, Egypt

Alsayed E. Mekky
Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo-11884, Egypt

Salem S. Salem
Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo-11884, Egypt, salemsalahsalem@azhar.edu.eg

How to Cite This Article

Zaher, Ahmed H.; Kabadaia, Mohammed M.; Hammad, Kotb M.; Mekky, Alsayed E.; and Salem, Salem S. (2023) "Forensic flies as carries of pathogenic bacteria associated with a pig carcass in Egypt," *Al-Azhar Bulletin of Science*: Vol. 34: Iss. 3, Article 5.
DOI: https://doi.org/10.58675/2636-3305.1657

ORIGINAL ARTICLE

Forensic Flies as Carries of Pathogenic Bacteria Associated with a Pig Carcass in Egypt

Ahmed H. Zaher [a], Mohammed M. Kabadaia [a], Kotb M. Hammad [a], Alsayed E. Mekky [b], Salem S. Salem [b,*]

[a] Zoology and Entomology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo-11884, Egypt
[b] Botany and Microbiology Department, Faculty of Science, AL-Azhar University, Nasr City, Cairo-11884, Egypt

Abstract

Investigations were conducted on the succession of forensic flies related to the stages of pig carcass decomposition, bacterial variety, and bacterial count associated with forensic flies' exterior body surfaces. Understanding the function of micro-organisms in the breakdown of carrion and how it pertains to post-mortem interval measurement depends on understanding that bacteria are the main decomposers. In place of human remains, pig-carcasses were used to monitor changes in the microbial community. This is a result of clinical investigations on humans frequently using pig corpses as animal models. Fresh and bloated phases attracted the biggest numbers and maximum diversity of fly species, according to the discovered bacterium species, which were decomposition stages were tallied. The initial fly species drawn to new and bloated pig carcasses were *Muscidae*, *Calliphoridae*, and *Sarcophagidea*. Bacterial species from *Chrysomya albiceps*, *Piophila casei*, Musca domestia, Sarcophage carrinaria, and Walfartia magnifica were isolated from their exterior body surfaces. The distribution of the presumed bacterial species, which varies in surface flies, includes *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Staphylococcus hominis*. The microbial population showed notable variance with respect to the time it took for decomposition.

Keywords: A pig carcass, Bacterial diversity, Decompositional stages, Flies succession

1. Introduction

The field of forensic entomology evaluates the biological and ecological features of the arthropod fauna that colonize the rhizosphere new areas. It belongs to the forensic-research field [1,2]. Animal carcasses supply us with a rich complex of insects that work together with bacteria to accelerate the de-composition process in the absence of vertebrate scavengers [3,4]. This study area can help determine the postmortem-interval (PM-I) by estimating the amount of time between death and body finding. The first insects to settle on a dead body are usually flies [5]. Although the specific fly species that are engaged vary depending on the locality, research have shown that the primary species are members of a very small number of families: Calliphorida, Sarcophagida, and Muscidae [6,7]. Insects are the first to identify and locate a body and are present in all phases of decomposition, and some species are unique to specific seasons and locations. This is the fundamental justification for using insects in criminal investigations, a science recognized as forensic entomology. The fact that ovulation might take place minutes after death is another crucial factor to take into account [6]. The initial stage of degradation of a dead body is the action of microorganisms like bacteria and fungus, which is then followed by a number of arthropods, with the predominance of sarcosa-prophagous insects [8,9]. The body changes naturally after death and goes through several stages of De-composition

Received 10 August 2023; revised 27 September 2023; accepted 10 October 2023.
Available online 30 November 2023

* Corresponding author. Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Nasr City, Cairo, 11884, Egypt.
E-mail address: salemsalahsalem@azhar.edu.eg (S.S. Salem).

https://doi.org/10.58675/2636-3305.1657

that are appealing to necrophagous insects [10]. To focus on changes in the bacterial populations, pig-carcass was utilized as mimics for human remain. This is due to the widespread use of pig-carcasses as experimental animals in clinical treatment investigations [11]. There are several characteristics of Degradation that are caused by bacteria (Fresh, bloat, active, advanced and skeletonization), hence they seems to manipulate the attitude of insects to inspire types that benefit their preservation while repelling those are detrimental to them [12,13]. The process of decay, the composition of the fauna, the order of succession, and the duration of insect growth are all influenced by various variables, including temperature, wind, rainfall, and geographic site. Therefore, the effects of the local fauna can be employed to determine the PMI when native climatological data are available [14]. Bacteria are primary decomposers, which is important for understanding the role of population in the breakdown process of carrion and how it relates to PMI measurement. In Bangalore, India, patterns of insect-abundance and distribution were investigated. The results suggested that despite their moderate size, residential gardens may be essential for sustaining urban insect biodiversity [15]. A number of micro-organisms were listed (primarily at the familial and generic ranks though inclusive of some species) that may be significant during bloat. In common, these authors record a shift in aerobic bacteria (*Staphylococcus* and *Enterobacteriacae*) to an-aerobic bacteria (*Clostridia* and *Bacteroides*) [16]. According to Janaway et al., autolysis and bacterial activity inside the tissues both contribute to the first dis-integration of tissue. Internal bacteria, such as those from the GI tract, spread as the tissues break down. Because of the lack of oxygenated blood, the tissue's loss of redox potential is probably what causes the transition from aerobic to an-aerobic organisms [17]. On an ecological investigation of fly-transmitted zoonotic-bacteria that are resistant to antibiotics in cattle ranches. In order to learn more about the function that flies may play as a possible vector for bacteria that are important zoonotic in cattle farms, they noted that both qualitative and quantitative monitoring of flies was done. Representative fly samples were cultivated to isolate bacteria, identify them biochemically, and test them against 12 common antibiotics [18]. Therefore, the goal of this work is to isolate, and characterize the bacteria present on the exterior bodies of forensic flies during the de-composition of pig-carcasses.

2. Materials and methods

2.1. Study site

The study's location was at University of Al-Azhar, Nasr City, Cairo, Egypt. In the botanic gardens where the animals were housed at the Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, pig-carcasses were placed in certain locations.

2.2. Experimental design

One pig-carcass (*Sus-scrofa domesticus* L) weighing ~10 kg was placed in a garden house. Pig-carcass was dead normally, and acquired of it from pig's farm at Almukatam, Cairo, Egypt. The carcass was immediately placed into mesh cages to prevent scavenging by large vertebrates and left exposed to natural condition. A hand net was used to catch flies. Identification and taxonomic determination of flies made by using current keys [19−22] and by specialists in Cairo University. Pig was visited each 3 h daily in order to determine the duration of decomposition stages. Images of pig throughout decomposition study were captured using digital Camera. Every day, the meteorology unit of kobri EL-Kobba in Cairo provided the ambient temperature and relative humidity.

2.3. Bacterial count

Serial dilutions of a subsample at each 5 sample suspension were prepared in sterile saline. Each dilution was then inoculated onto 2 plates of plate-count ager and incubated overnight at 37 °C. Colony-forming units (CFU/mL) were then counted so that the total numbers of bacteria returned from the exterior surface of each fly could be estimated. Briefly, Forensic flies individually were shaken thoroughly in sterile saline-solution (2 ml) for 2 min. The suspension was then serially dilute and inoculated on plate count Agar. At 37 °C, plates were left incubating for 24 h [23].

2.4. Bacterial isolation and identification

Various bacteria were isolated from the forensic flies' exterior body by using the normal isolation technique. On fresh blood agar plates, bacterial colonies displaying morphological variations were selected and streaked. Following daily growth

monitoring, all bacteria colonies were subculture onto the appropriate media and incubated once more until pure colonies were produced. According to Bergys' handbook of systematic-bacteriology [24], the bacteria were identified down to the genus level using morphological, physiological, and biochemical testing. This identification was then validated using the Biameriux vitek-2 system.

3. Results

3.1. Climatic conditions

Ambient temperatures and relative humidity for pig placed in abotanical garden of the animal house were obtained from meteorology unit, Egypt during the study period. The min. and max. temperatures were varied from 43 °C to 20 °C with an average of 32 °C. While the relative humidity was varied from 85 to 12 % with an average of 48 % (Table 1, Figs. 1 and 2).

3.2. Decomposition stages of pig carcass

The fresh stage of pig-carcass began with death and ended when bloating was initiated. The fresh-stage lasted from zero to day 01 postmortem. The beginning of bloated stage was on day 2 Postmortem. The end of the bloated stage and beginning of the active decay stage was evidence of liquefaction. Evidence of liquefaction first occurred on day four; the advanced decay stages began when flesh of pig-carcass removed at head, limbs and anus. This stage arrived on day seven. The dry stage, which is the last stage of de-composition, is distinguished by little or no odor, hardened, dried, exposed bone. This stage was arrived on day 21 (Table 2 and Fig. 3).

3.3. Succession of forensic flies associated with compositional stages of pig carcass

A total of 491 flies representing, 5 species and 4 families (Table 3) were collected during decomposition stages of pig carcass.

3.3.1. Fresh stage

From pig carcasses at the fresh stage, one species of adult Muscidae, *Musca domestica*, was identified (2). *W. magnifica*, a species from the family Sarcophagidae, has a variety of (1).

Table 1. Climatic information gathered throughout the experiment from 25 July to 1 September 2021.

Temperature (°C)			Relative humidity (%)		
Max.	Min.	Average	Max.	Min.	Average
43	20	32	85	12	48

3.3.2. Bloated stage

One species of adult Calliphorida was collected from pig carcasss during bloated stage, namely, *Chrysomya albiceps* with total number of (121). Family Muscidae was represented by one species namely, *M. domestica* with total number of (103). Family Sarcophagida was represented by 2 species namely, *Sarcophaga carnaria* (16), and *W. magnifica* (6). *Piophila casei* (Fam. Piophilidae) was with number (12).

3.3.3. Decay stage

Two species of adult Sarcophagida were collected from pig carcasss during decay stage, the most frequent species were, *W. magnifica* (4), and. *carnaria* (7). From family Calliphoridae one species was collected namely, *C. albiceps* with a number of (102), while family Muscidae was represented by only *M. domestica* with total number of (115). *P. casei* (Fam. Piophilidae) was with number (2). From the aforementioned results, it is appeared that pig carcass attracted the greater numbers and higher diversity of forensic flies during fresh, bloated and decay stages. It was interesting to note the advanced decay and the dry stages of pig-carcass were distinguished by the absence of flies.

3.4. Bacterial diversity

The microbial load from samples underneath decomposing pig carrion using conventional methods revealed Gram-positive and Gram-negative aerobic-bacteria (Table 4). This demonstrates how bacteria participating in decomposition produce a variety of environments and microhabitats.

3.5. Identification of bacteria

Samples yielded 27 different bacterial isolates by agar streaking method onto surface plates of nutrient agar, these nutrient agar plates used as standard control for the growth and colonial characters and blood agar media and then the plates were incubated aerobically and an anerobically at 37 °C for 24 h, after growth, bacterial colonies were subjected to purification processes by inoculating on different selective media (Table 5). After incubation, different colonies that appear from different specimens were selected for the primary identification which based on morphological and biochemical parameters. In the current study 27 isolates were harvested from samples and grown on different media, all isolates were

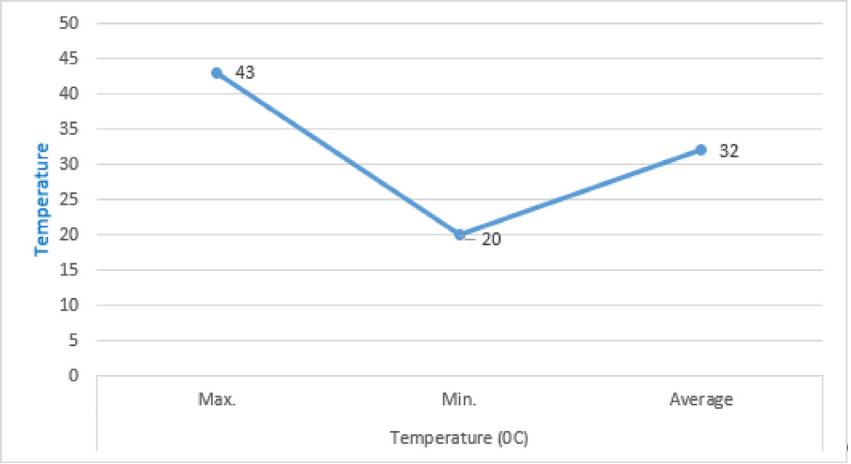

Fig. 1. Ambient temperature during the study period from 25 July to 1 September 2021.

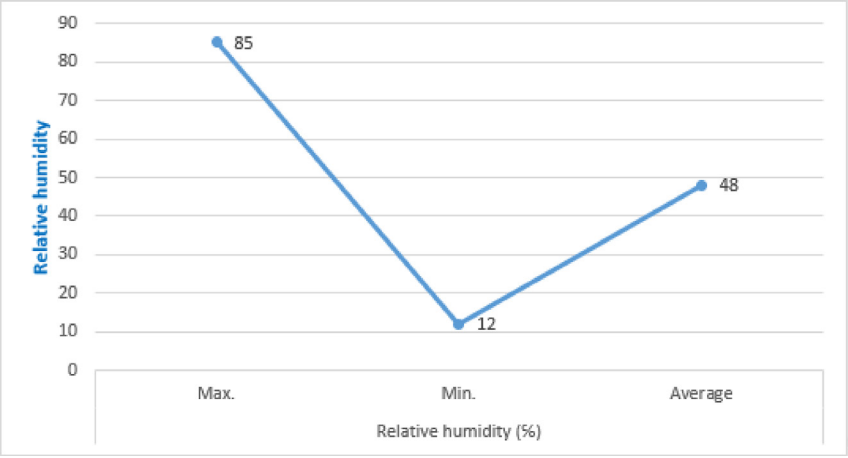

Fig. 2. Relative humidity during the study period from 25 July to 1 September 2021.

grown normally on nutrient agar and blood agar, while 14 of 27 able to grow on MacConkey agar medium, 7 isolates only grown on mannitol agar medium. According to gram stain reaction 14 isolates were negatively reactive with gram stain while others 13 g positive.

Numerous bacterial species, mostly enteric bacteria, are linked to the biodegradation of bodily tissues. The distribution of the presumed aerobic bacterial species, which vary in decomposition fluid, includes *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis,* and *Staphylococcus hominis*. The microbial population showed notable variance with respect to the time it took for decomposition, according to this study Table 6 and confirmed this identification by using Biameriux vitek-2 system (Tables 7–13).

4. Discussion

The exterior body surface of *C. albiceps* was surveyed and noted during the decomposition stages of a pig-carcass for the current investigation. We found

Table 2. Decomposition stages of pig-carcass from 25 July to 1 September 2021.

Decomposition stages	Days of postmortem	Temperature (°C)			Relative humidity (%)		
		Max.	Min.	Average	Max.	Min.	Average
Fresh	0–1	34	26	30	70	23	47
Bloated	2–3	35	25	30	67	20	44.5
Active decay	4–6	38	26	31	69	17	41
Advanced decay	7–20	43	20	33	73	12	40.2
Dry	21–36	37	24	30.7	85	25	54.3

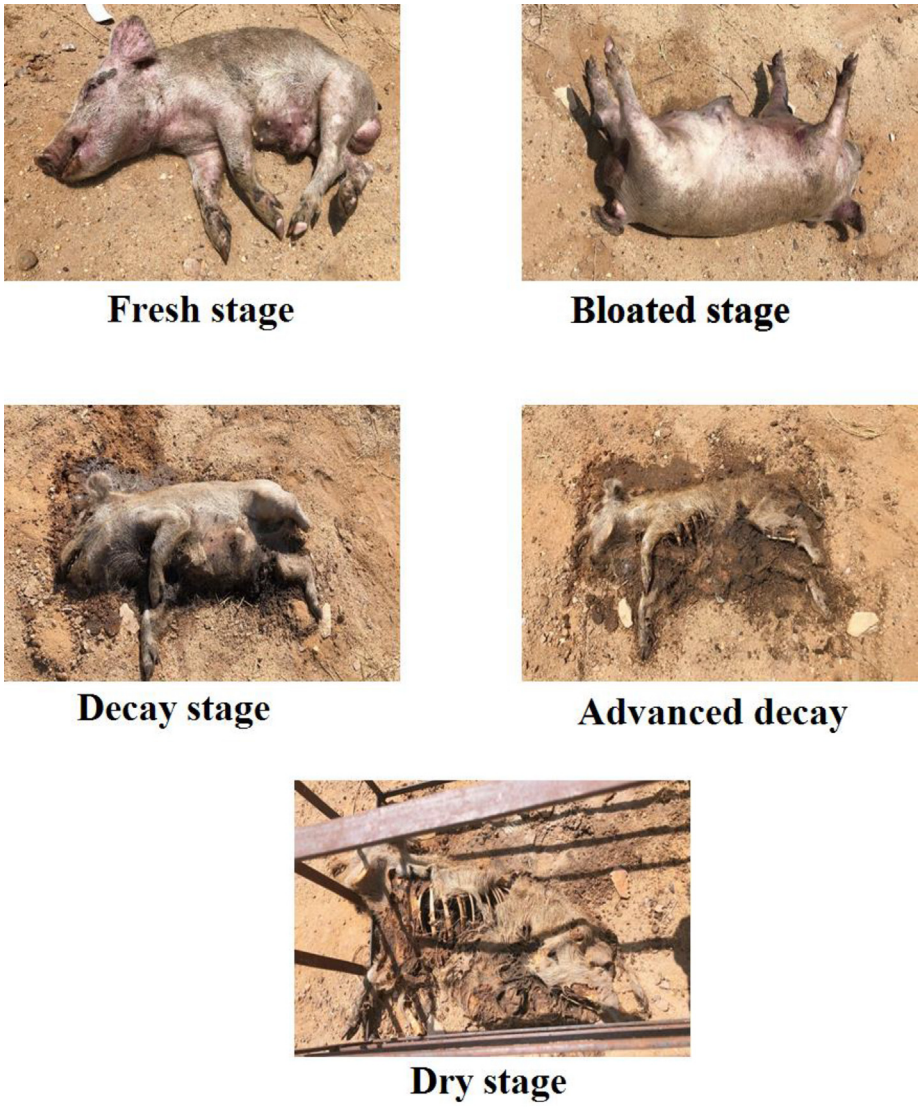

Fig. 3. Compositional stages of pig-carcass during study period from 25 July to 1 September 2021.

Table 3. Forensic flies' succession associated with decomposionnal stages of pig-carcass.

Decomposition stages of pig carcass	Families/species of flies					
	Calliphoridae	Muscidae	Piophilidae	family Sarcophagidae		
	Chrysomyaalbiceps	*Musca domestica*	*Piophila casei*	*Sarcophaga carnaria*	Wohlfartia magnifica	Total
Fresh	0	2	0	0	1	3
Bloated	121	103	12	16	6	258
Decay	102	115	2	7	4	230
Advanced decay	0	0	0	0	0	0
Dry	0	0	0	0	0	0
Total						491

that absence of *C. albiceps* in initial stage and display in both Bloated and Decay stages. Another study more insects were harvested from carcasses exposed to the sun, while a larger number of specimens were reared from those in the shade. The stages of pig degradation as well as insect behavior and abundance were controlled by temp and rainfall. In all four tests, *C. albiceps* was the most prevalent species [10]. During the pig-carcass' decomposition, strains of bacteria were discovered and noted from the

Table 4. Bacterial counts during the decomposition period.

No.	Day of sampling	Specimen Code	Dilution used	Number of colonies	Total bacterial count (CFU/G)
1	25/2/2021	W25	10^{-3}	67	67×10^{-3}
2	25/2/2021	C25-1	10^{-3}	200	200×10^{-3}
3	25/2/2021	C25-2	10^{-3}	132	132×10^{-3}
4	25/2/2021	C25-3	10^{-3}	155	155×10^{-3}
5	26/2/2021	S26-1	10^{-3}	125	125×10^{-3}
6	26/2/2021	S26-2	10^{-3}	148	148×10^{-3}
7	26/2/2021	S26-3	10^{-3}	154	154×10^{-3}
8	26/2/2021	W26	10^{-3}	156	156×10^{-3}
9	26/2/2021	C26-1	10^{-3}	125	125×10^{-3}
10	26/2/2021	C26-2	10^{-3}	147	147×10^{-3}
11	27/2/2021	M27	10^{-3}	158	158×10^{-3}
12	27/2/2021	S27-1	10^{-3}	158	158×10^{-3}
13	27/2/2021	S27-2	10^{-3}	169	169×10^{-3}
14	27/2/2021	S27-3	10^{-3}	187	187×10^{-3}
15	28/2/2021	M28-1	10^{-3}	145	145×10^{-3}
16	28/2/2021	M28-2	10^{-3}	154	154×10^{-3}
17	28/2/2021	M28-3	10^{-3}	125	125×10^{-3}
18	28/2/2021	M28-4	10^{-3}	187	187×10^{-3}
19	25/2/2021	C28-1	10^{-3}	16	16×10^{-3}
20	28/2/2021	C28-2	10^{-3}	148	148×10^{-3}
21	28/2/2021	S28-1	10^{-3}	200	200×10^{-3}
22	28/2/2021	S28-2	10^{-3}	123	123×10^{-3}
23	28/2/2021	S28-3	10^{-3}	147	147×10^{-3}
24	28/2/2021	S28-4	10^{-3}	158	158×10^{-3}
25	1/3/2021	B1-1	10^{-3}	169	169×10^{-3}
26	1/3/2021	B1-2	10^{-3}	145	145×10^{-3}
27	1/3/2021	B1-3	10^{-3}	169	169×10^{-3}

Table 5. Primary identification of bacterial-isolates.

No.	Specimen Code	Nutrient Agar	Blood Agar	MacConky Agar	Manitol Agar	Gram Stain	KOH Test
1	**W25**	+	+	−	+	+	−
2	**C25-1**	+	+	+	−	−	+
3	**C25- 2**	+	+	+	−	−	+
4	**C25-3**	+	+	+	−	−	+
5	**S26-1**	+	+	+	−	−	+
6	**S26-2**	+	+	+	−	−	+
7	**S26-3**	+	+	+	−	−	+
8	**W26**	+	+	−	+	+	−
9	**C26-1**	+	+	−	+	+	−
10	**C26-2**	+	+	−	+	+	−
11	**M27**	+	+	−	+	+	−
12	**S27-1**	+	+	+	−	−	+
13	**S27-2**	+	+	+	−	−	+
14	**S27-3**	+	+	+	−	−	+
15	**M28-1**	+	+	−	+	+	−
16	**M28-2**	+	+	−	+	+	−
17	**M28-3**	+	+	−	+	+	−
18	**M28-4**	+	+	−	+	+	−
19	**C28-1**	+	+	−	+	+	−
20	**C28-2**	+	+	−	+	+	−
21	**S28-1**	+	+	+	−	−	+
22	**S28-2**	+	+	+	−	−	+
23	**S28-3**	+	+	+	−	−	+
24	**S28-4**	+	+	+	−	−	+
25	**B1-1**	+	+	+	−	−	+
26	**B1-2**	+	+	−	+	+	−
27	**B1-3**	+	+	−	+	+	−

Table 6. Identification of bacterial isolates.

Test code	Motility	Catalase	Oxidase	sugar Fermentation	Nitrate Reduction	Methyl Red	Vogues Proskour	Indole Production	Citrate Utilization	H2S Production	Urease	Coagulase	Suspected Organism
W25-6	−	+	−	+	+	+	+	−	+	−	+	−	*Staphylococcus epidermidis*
C25-1	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
C25-2	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
C25-3	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
S26-1	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
S26-2	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
S26-3	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
W26	−	+	−	+	+	+	+	−	+	−	+	−	*Staphylococcus aureus*
C26-1	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
C26-2	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
M27	−	+	−	+	+	+	+	−	+	−	+	−	*Staphylococcus aureus*
CH26-1	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
CH26-2	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
CH26-3	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
CH27-1	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
CH27-2	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
CH27-3	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
S27-1	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
S27-2	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
S27-3	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
CH28-1	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
CH28-2	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
CH28-3	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
M28-1	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
M28-2	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
M28-3	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
M28-4	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
C28-1	−	+	−	+	+	+	+	−	+	−	+	−	*Staphylococcus hominis*
C28-2	−	+	−	+	+	+	+	−	+	−	+	−	*Staphylococcus aureus*
S28-1	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
S28-2	+	+	+	+	+	+	−	+	+	−	+	−	*Pseudomonas aeruginosa*
S28-3	−	+	−	−	−	+	−	−	+	−	−	−	*Proteus mirabilis*
S28-4	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
B1-1	+	+	−	+	+	+	−	+	−	−	−	−	*Escherichia coli*
B1-2	−	+	−	+	+	+	+	−	+	−	−	+	*Bacillus subtilis*
B1-3	−	+	−	+	+	+	+	−	+	−	+	−	*Staphylococcus aureus*

Table 7. Identification information for Staphylococcus epidermidis using VITEK2 system.

No.	Probability			Card			Confidence			Analysis time			organism				
1	99 %			Gp			Very good Identification			5:15 h.			*Staphylococcus epidermidis*				
Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result
1	AMY	−	9	PIPLC	−	17	AspA	−	25	AGAL	−	33	POLYB	−	41	BACi	+
2	APPA	−	10	CDEX	−	18	BGURr	−	26	URE	+	34	dMAL	+	42	PUL	−
3	LeuA	−	11	ProA	−	19	Dsor	−	27	NAG	−	35	MBdG	−	43	ADH2S	−
4	ALaA	−	12	TYrA	−	20	LAC	−	28	dMNE	−	36	dTRE	+	44		
5	dRIB	−	13	ILATK	+	21	dMAN	+	29	SAC	+	37	AGLU	−	45		
6	NOVO	−	14	NC6.5	+	22	SAL	−	30	AMAN	−	38	BGUR	−	46		
7	Draf	−	15	O129R	+	23	ADH1	−	31	BGAL	−	39	PHOS	−	47		
8	OPTO	+	16	Dxyl	−	24	BGAR	−	32	PyrA	−	40	dGAL	+	48		

Table 8. Identification information for Escherichia coli using VITEK2 system.

No.	Probability			Card			Confidence			Analysis time			organism				
2	95 %			GN			Very good Identification			5:25 h.			*Escherichia coli*				
Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result
1	APPA	−	9	ADO	+	17	PyrA	−	25	IARL	−	33	dCEL	−	41	BGAL	+
2	H2S	−	10	BNAG	−	18	AGLTp	−	26	dGLU	+	34	GGT	−	42	OFF	+
3	BGLU	−	11	dMAL	+	19	dMAN	+	27	dMNE	+	35	BXYL	−	43	BAIap	−
4	ProA	+	12	LIP	−	20	PLE	−	28	TyrA	+	36	URE	−	44	dSOR	+
5	SAC	+	13	dTAG	−	21	dTRE	+	29	CIT	−	37	MNT	−	45	5 KG	−
6	ILATk	−	14	AGLU	−	22	SUCT	+	30	NAGA	−	38	AGAL	+	46	PHOS	−
7	GlyA	+	15	ODC	−	23	LDC	+	31	IHISa	−	39	CMT	+	47	BGUR	+
8	0129R	+	16	GGAA	−	24	MLTa	−	32	ELLM	−	40	ILATa	−	48		

Table 9. Identification information Staphylococcus aureus using VITEK2 system.

No.	Probability			Card			Confidence			Analysis time			organism				
3	97 %			Gp			Very good Identification			5:25 h.			*Staphylococcus aureus*				
Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result
1	AMY	−	9	PIPLC	−	17	AspA	−	25	AGAL	−	33	POLYB	−	41	BACi	+
2	APPA	−	10	CDEX	−	18	BGURr	−	26	URE	+	34	dMAL	+	42	PUL	−
3	LeuA	−	11	ProA	−	19	Dsor	−	27	NAG	−	35	MBdG	−	43	ADH2S	−
4	ALaA	−	12	TYrA	−	20	LAC	−	28	dMNE	−	36	dTRE	+	44		
5	dRIB	−	13	ILATK	+	21	dMAN	+	29	SAC	+	37	AGLU	−	45		
6	NOVO	−	14	NC6.5	+	22	SAL	−	30	AMAN	−	38	BGUR	−	46		
7	Draf	−	15	O129R	+	23	ADH1	−	31	BGAL	−	39	PHOS	−	47		
8	OPTO	+	16	Dxyl	−	24	BGAR	−	32	PyrA	−	40	dGAL	+	48		

Table 10. Identification information for Bacillus subtilis using VITEK2 system.

No.	Probability			Card			Confidence			Analysis time			organism				
4	95 %			GN			Very good Identification			5:25 h.			*Bacillus subtilis*				
Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result
1	AMY	−	9	PIPLC	−	17	AspA	−	25	AGAL	−	33	POLYB	−	41	BACi	+
2	APPA	−	10	CDEX	−	18	BGURr	−	26	URE	+	34	dMAL	+	42	PUL	−
3	LeuA	−	11	ProA	−	19	Dsor	−	27	NAG	−	35	MBdG	−	43	ADH2S	−
4	ALaA	−	12	TYrA	−	20	LAC	−	28	dMNE	−	36	dTRE	+	44		
5	dRIB	−	13	ILATK	+	21	dMAN	+	29	SAC	+	37	AGLU	−	45		
6	NOVO	−	14	NC6.5	+	22	SAL	−	30	AMAN	−	38	BGUR	−	46		
7	Draf	−	15	O129R	+	23	ADH1	−	31	BGAL	−	39	PHOS	−	47		
8	OPTO	+	16	Dxyl	−	24	BGAR	−	32	PyrA	−	40	dGAL	+	48		

Table 11. Identification information for Staphylococcus hominis using VITEK2 system.

No	Probability			Card			Confidence			Analysis time			organism				
5	95 %			GN			Very good Identification			5:25 h.			*Staphylococcus hominis*				
Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result
1	AMY	−	9	PIPLC	−	17	AspA	−	25	AGAL	−	33	POLYB	−	41	BACi	+
2	APPA	−	10	CDEX	−	18	BGURr	−	26	URE	+	34	dMAL	+	42	PUL	−
3	LeuA	−	11	ProA	−	19	Dsor	−	27	NAG	−	35	MBdG	−	43	ADH2S	−
4	ALaA	−	12	TYrA	−	20	LAC	−	28	dMNE	−	36	dTRE	+	44		
5	dRIB	−	13	ILATK	+	21	dMAN	+	29	SAC	+	37	AGLU	−	45		
6	NOVO	−	14	NC6.5	+	22	SAL	−	30	AMAN	−	38	BGUR	−	46		
7	Draf	−	15	O129R	+	23	ADH1	−	31	BGAL	−	39	PHOS	−	47		
8	OPTO	+	16	Dxyl	−	24	BGAR	−	32	PyrA	−	40	dGAL	+	48		

Table 12. Identification information for Pseudomonas aeruginosa using VITEK2 system.

No.	Probability			Card			Confidence			Analysis time			organism				
6	95 %			GN			Very good Identification			5:25 h.			*Pseudomonas aeruginosa*				
Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result
1	APPA	−	9	ADO	+	17	PyrA	−	25	IARL	−	33	dCEL	−	41	BGAL	+
2	H2S	−	10	BNAG	−	18	AGLTp	−	26	dGLU	+	34	GGT	−	42	OFF	+
3	BGLU	−	11	dMAL	+	19	dMAN	+	27	dMNE	+	35	BXYL	−	43	BAIap	−
4	ProA	+	12	LIP	−	20	PLE	−	28	TyrA	+	36	URE	−	44	dSOR	+
5	SAC	+	13	dTAG	−	21	dTRE	+	29	CIT	−	37	MNT	−	45	5 KG	−
6	ILATk	−	14	AGLU	−	22	SUCT	+	30	NAGA	−	38	AGAL	+	46	PHOS	−
7	GlyA	+	15	ODC	−	23	LDC	+	31	IHISa	−	39	CMT	+	47	BGUR	+
8	0129R	+	16	GGAA	−	24	MLTa	−	32	ELLM	−	40	ILATa	−	48		

Table 13. Identification information for Proteus mirabilis using VITEK2 system.

No.	Probability			Card			Confidence			Analysis time			organism			
7	95 %			GN			Very good Identification			5:25 h.			*Proteus mirabilis*			
Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result	Number	Test	Result
1	APPA	−	9	ADO	+	17	PyrA	−	25	IARL	−	33	dCEL	−	41	BGAL	+
2	H2S	−	10	BNAG	−	18	AGLTp	−	26	dGLU	+	34	GGT	−	42	OFF	+
3	BGLU	−	11	dMAL	+	19	dMAN	+	27	dMNE	+	35	BXYL	−	43	BAIap	−
4	ProA	+	12	LIP	−	20	PLE	−	28	TyrA	+	36	URE	−	44	dSOR	+
5	SAC	+	13	dTAG	−	21	dTRE	+	29	CIT	−	37	MNT	−	45	5 KG	−
6	ILATk	−	14	AGLU	−	22	SUCT	+	30	NAGA	−	38	AGAL	+	46	PHOS	−
7	GlyA	+	15	ODC	−	23	LDC	+	31	IHISa	−	39	CMT	+	47	BGUR	+
8	0129R	+	16	GGAA	−	24	MLTa	−	32	ELLM	−	40	ILATa	−	48		

outer body layer of *M. domestica*. We found that *M. domestica* are present in all stages of decaying. According to Mabika et al. [25], the sun-exposed pig included members of the five (5) Dipteran families Muscidae, Calliphoridae, Sarcophagidae, Drosophilidae, and Phoridae. The strains that were gathered included *Drosophila* sp., *Sarcophaga* sp., *M. domestica*, *L.cuprina*, and *C.albiceps*. The pig in the shaded area belonged to four different families: Anthomyiidae, Calliphoridae, Phoridae, and Muscidae. *Hydrotaea* sp., *Lucilia cuprina*, *M. domestica*, and *C. albiceps* were cases of these families. Both corpses contained members of the three Coleopteran families Cleridae, Histeridae, and Dermestidae. *Lucilia* sp, *L.cuprina*, *Sarcophaga* sp., *C. albiceps*, and *Sepsis* sp. were among the flies came out of the raising units. During the decomposition of a pig-carcass for the current experiment, *W. magnifica's* exterior body surface was recovered and noted. We found that *W. magnifica* are present only in initial and bloated stages and not found in decaying stage. Matuszewski et al. [26] working on pig putted in shaded and sun sites, and in opens and forest habitats, respectively. In addition, habitat variations affected species diversity. Outdoor (sun-exposed) carcasses attracted a wider variety insect types and more individuals of each types, compared with indoor (Shaded) carcasses, they collect about 68 (Wohlfartia magnifica adult specimens). Bacterial were identified and noted from the exterior body of *S. carnaria* during the pig-carcass' decomposition. We found that *S. carnaria* found only in decaying stage. Matuszewski et al. [26] working on pig carrion collect about 91 (*Sarcophaga carnaria* adult specimens). Bacterial were identified and noted from the exterior body of *P. casei* during the pig-carcass' decomposition. *P. casei* was absent in the fresh stage but was present in the bloated and decay stages, respectively. Matuszewski et al. [26] working on pig collect about 462 (*Piophila casei* adult specimens).Various types of bacteria are accompanied with bio-degradation of body-cell tissues. Table 6 lists the microorganisms that are thought to be present with the carrion as it decomposes. The bacteria that were identified are *B. subtilis, S. aureus, S. epidermidis, E. coli, S. hominis, P. aeruginosa* and *P. mirabilis*, this corroborates the findings of Hopkins [27] and According to Pascual et al. [28], intestinal soil bacteria play a significant role in the decomposition of animal tissues. According to Kaiko and Stappenbeck [29], successful breakdown of animal tissues to obtain nutrients for development requires complex microbial interactions and successions, which can be impacted by environmental factors. This supports the findings of Finley et al. [30], who

found that when accumulated gas and fluid are expelled from the skin, breakdown elements emerge from the internal environment into the external environment. Insects and microbes repeatedly engage in persistent activity during this period, which is followed by an active decay. Among staphylococcal species, *S. aureus* has been shown as a clinically relevant human pathogen, and in many individuals it may lead to asymptomatic colonization [31]. According to other studies, nares-only detection can overestimated the frequency of *S. aureus* colonisation, and accurate determination requires sampling from other sites of body [32]. The process of recycling carbon, nitrogen, and sulphur into forms that plants can absorb is mostly carried out by bacteria. For instance, heterotrophic bacteria such as *Bacillus* break down proteins to release ammonia, which other bacteria then oxidize to produce nitrogen dioxide and ultimately nitrate. Using nitrate as an import of nitrogen, plants can breakdown it [33]. Immediately after death, the body's digestive enzymes and other chemicals start chemically modifying and degrading the remains (autolysis). Microbial communities, from the exterior surface and the gastro-intestinal tract, which are kept in check while the animal is alive, are also free to initiate and participate in the de-composition of the animal. Putrefaction is the proliferation of bacteria, from within the layer-body after death [28]. The foul smell of these chemicals and the body's coloring (blue, yellow, black, green, and red) as the tissue deteriorates serve as visual cues to this process. The bacteria also release significant amounts of gases through fermentation as they break down the pig carcass. Volatile compounds produced by fermentation draw numerous invertebrates and vertebrates that aid in the de-composition of the remains [34].

4.1. Conclusion

By utilizing expected changes in the microbial and arthropod community structure, the newly developing area of forensic biology has sought to address several issues that arise when calculating post-mortem interval. The corpses of pig is frequently employed in clinical human investigations as animal models. The number of bacterial species isolated from the exterior body surface from *C. albiceps*, *P. casei*, *Musca domestia*, *Sarcophage carrinaria* and *Walfartia magnifica*. The high bacterial diversity were isolated from *S. carrinaria* and *P. casei* during decaying decomposition stage. In order to apply forensic biology to identify the cause of death, abuse, or neglect, this study focuses on the types of bacterial-communities that occur during the de-composition process.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Funding

Not applicable.

Ethics approval

In the experiment care was followed the CITES no. 123 of 18 March 1986 and 2005 revision of the European convention for the protection of vertebrate animal used for experimental and other scientific purposes and the Commission Recommendation of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes (C(2007)2525:http://ec.europa.eu/transparency/regdoc/rep/3/2007/EN/3-2007- 2525-EN-1-0.Pdf).

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Authors' contributions

Conceptualization: M. M. K., K. M. H., and S. S. S.; Methodology and Resources: A. H. Z., M. M. K., K. M. H., A. E. M., and S. S. S.; Writing—Original Draft Preparation: A. H. Z., M. M. K., K. M. H., A. E. M., and S. S. S.; Writing—Review and Editing: A. H. Z., M. M. K., K. M. H., A. E. M., and S. S. S.; Supervision: M. M. K., K. M. H., and S. S. S.. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

The authors express their sincere thanks to Faculty of science (Boyes), Al-Azhar University, Cairo, Egypt for providing the necessary research facilities.

References

[1] Byrd JH, Tomberlin JK. Forensic entomology: the utility of arthropods in legal investigations. CRC Press; 2019.
[2] Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, Hall M JR. Best practice in forensic entomology—standards and guidelines. Int J Leg Med 2007;121:90−104.
[3] Michaud J-P, Majka CG, Privé J-P, Moreau G. Natural and anthropogenic changes in the insect fauna associated with carcasses in the North American Maritime lowlands. Forensic Sci Int 2010;202:64−70.
[4] Wang Y, Ma M-Y, Jiang X-Y, Wang J-F, Li L-L, Yin X-J, et al. Insect succession on remains of human and animals in Shenzhen, China. Forensic Sci Int 2017;271:75−86.
[5] Lee Goff M. Early post-mortem changes and stages of decomposition in exposed cadavers. Exp Appl Acarol 2009; 49:21−36.
[6] Smith K. A manual of forensic entomology. Trustees of the British Museum. London: Natural History and Cornell University Press; 1986. p. 205.
[7] Eberhardt TL, Elliot DA. A preliminary investigation of insect colonisation and succession on remains in New Zealand. Forensic Sci Int 2008;176:217−23.
[8] Nuorteva P. Sarcosaprophagous insects as forensic indicators. Forensic Medicine : A Study in Trauma and Environmental Hazards 1977;2:1072−95.
[9] Dash HR, Das S. Microbial degradation of forensic samples of biological origin: potential threat to human DNA typing. Mol Biotechnol 2018;60:141−53.
[10] Carvalho LMLD, Thyssen PJ, Linhares AX, Palhares FAB. A checklist of arthropods associated with pig carrion and human corpses in Southeastern Brazil. Mem Inst Oswaldo Cruz 2000;95:135−8.
[11] Kyparos A, Feeback DL, Layne CS, Martinez DA, Clarke MS. Mechanical stimulation of the plantar foot surface attenuates soleus muscle atrophy induced by hindlimb unloading in rats. J Appl Physiol 2005;99:739−46.
[12] Campobasso CP, Di Vella G, Introna F. Factors affecting decomposition and Diptera colonization. Forensic Sci Int 2001;120:18−27.
[13] Singh B, Crippen TL, Zheng L, Fields AT, Yu Z, Ma Q, et al. A metagenomic assessment of the bacteria associated with Lucilia sericata and Lucilia cuprina (Diptera: Calliphoridae). Appl Microbiol Biotechnol 2015;99:869−83.
[14] Battán Horenstein M, Rosso B, García MD. Seasonal structure and dynamics of sarcosaprophagous fauna on pig carrion in a rural area of Cordoba (Argentina): their importance in forensic science. Forensic Sci Int 2012;217:146−56.
[15] Jaganmohan M, Vailshery LS, Nagendra H. Patterns of insect abundance and distribution in urban domestic gardens in Bangalore, India. Diversity 2013;5:767−78.
[16] Förster M, Klimpel S, Mehlhorn H, Sievert K, Messler S, Pfeffer K. Pilot study on synanthropic flies (e.g. Musca, Sarcophaga, Calliphora, Fannia, Lucilia, Stomoxys) as vectors of pathogenic microorganisms. Parasitol Res 2007;101: 243−6.
[17] Janaway RC, Percival SL, Wilson AS. Decomposition of human remains. In: Percival SL, editor. Microbiology and aging: clinical manifestations. Totowa, NJ: Humana Press; 2009. p. 313−34.
[18] Mohammed AN, Abdel-Latef GK, Abdel-Azeem NM, El-Dakhly KM. Ecological study on antimicrobial-resistant zoonotic bacteria transmitted by flies in cattle farms. Parasitol Res 2016;115:3889−96.
[19] Mosallam S. Biological studies of some myiasis producing dipterous flies in Cairo. Ph. D. Thesis. Cairo: Ain Shams University; 1980.
[20] Shaumar NF, Mohammed SK, Mohammed SA. Keys for identification of species of family Calliphoridae (Diptera) in Egypt. J Egypt Soc Parasitol 1989;19:669−81.
[21] Whitworth T. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. Byrd JH. In: Tomberlin JK, editor. Forensic entomology: the utility of arthropods in legal investigations. 3rd ed. 2006. p. 413−43.
[22] Carvalho CJBD, Mello-Patiu CAD. Key to the adults of the most common forensic species of Diptera in South America. Rev Bras Entomol 2008;52:390−406.
[23] Fouda A, Fouda MA, Aldally AG, Ismael SB, Awad MA. Isolation and identification of bacterial species associated with non-biting flies in Egypt. Egypt. Acad. J. Biol. Sci 2016;9: 37−45.
[24] Ludwig W, Schleifer K-H, Whitman WB. Revised road map to the phylum Firmicutes. In: De Vos P, Garrity GM, Jones D, et al., editors. Bergey's Manual® of systematic bacteriology: volume three the firmicutes. New York, NY: Springer; 2009. p. 1−13.
[25] Mabika N, Masendu R, Mawera G. An initial study of insect succession on decomposing rabbit carrions in Harare, Zimbabwe. Asian Pac J Trop Biomed 2014;4:561−5.
[26] Matuszewski S, Szafałowicz M, Jarmusz M. Insects colonising carcasses in open and forest habitats of Central Europe: search for indicators of corpse relocation. Forensic Sci Int 2013;231:234−9.
[27] Hopkins DW. The role of soil organisms in terrestrial decomposition. In: Tibbett M, Carter DO, editors. Soil analysis in forensic taphonomy. CRC Press; 2008. p. 65−78.
[28] Pascual J, von Hoermann C, Rottler-Hoermann A-M, Nevo O, Geppert A, Sikorski J, et al. Function of bacterial community dynamics in the formation of cadaveric semiochemicals during in situ carcass decomposition. Environ Microbiol 2017;19:3310−22.
[29] Kaiko GE, Stappenbeck TS. Host−microbe interactions shaping the gastrointestinal environment. Trends Immunol 2014;35:538−48.
[30] Finley SJ, Pechal JL, Benbow ME, Robertson BK, Javan GT. Microbial signatures of cadaver gravesoil during decomposition. Microb Ecol 2016;71:524−9.
[31] Esmailnezhad Z, Haghkhah M, Ghaffari N. Coagulase gene heterogeneity in bovine mastitic S. aureus isolates. Online J Vet Res 2012;16:53−61.
[32] Gould IM, David MZ, Esposito S, Garau J, Lina G, Mazzei T, et al. New insights into meticillin-resistant Staphylococcus aureus (MRSA) pathogenesis, treatment and resistance. Int J Antimicrob Agents 2012;39:96−104.
[33] Spennemann DH, Franke B. Decomposition of buried human bodies and associated death scene materials on coral atolls in the tropical Pacific. J Forensic Sci 1995;40:356−67.
[34] DeVault TL, Rhodes J, Olin E, Shivik JA. Scavenging by vertebrates: behavioral, ecological, and evolutionary perspectives on an important energy transfer pathway in terrestrial ecosystems. Oikos 2003;102:225−34.