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ISOLATION AND IDENTIFICATION OF SOME OF BACTERIAL PATHOGENS FROM FLIES IN THE ANIMAL FARM

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Abstract

This study was carried out to investigate and isolate the bacterial pathogens from flies' samples of animal farm. Sixty flies' samples were collected randomly from animal farm in the college of veterinary medicine / Baghdad University during November 2018- December 2018. Flies samples were transport to the laboratory then treated by two procedures. The first one is to wash these flies sporadically to gain the surface bacterial flora and the second one is homogenized the flies sporadically to gain the internal bacterial flora. Then, the fluid that gained from these two procedures cultivated on blood and nutrient agar. Then gram staining was done to determine these bacteria if they are gram positive or negative. These microorganisms cultured on specific media and incubated at 37°C for 24hrs to gain pure colonies. Then the isolated bacteria were identified by culture characteristic features and different biochemical tests. The results were showed that the obtained isolates including *E. coli* were 46.6% and 66.6% in surface and internal flora respectively. Whereas *Klebsiella* isolates were 6.6% and 15% in surface and internal flora, respectively. Whereas, *Salmonella* isolates were 6.6% and 5% in surface and internal flora respectively. Meanwhile, *Pseudomonas* isolates were 3.3% in surface flora only, *Streptococcus* and *Stapohylococcus* isolates were 50% and 20% in surface flora. *Proteus* isolate was 6.6% from internal flora. In conclusion the study indicated that flies play a significant role in transmits of many significant bacterial pathogens, which cause many important diseases to humans and animals therefore must pay attention and make efforts to eliminate its effect.

Key words : Bacterial pathogens, flies, surface, internal floras.

Introduction

The housefly, *Musca domestica* is mainly common habitant fly species, which have been seen in dumps, domestic waste bins and other areas of poor hygienic conditions. Houseflies enter a number of places, including contaminated places due to their own biologic habits for feeding (Service, 2000). It has been known as an significant medical insect all around the world (Graczyk *et al*., 2001) and it is not only a annoyance pest but also acts as a vital vector for lots of pathogenic microorganism including bacteria among humans and animals (Hussein and John, 2017; Zurek and Gorham, 2010). Excessive fly populations are annoying to inhabitants and farm workers when these are nearby to human habitations to create public health problems (Sarwar, 2016). Houseflies convey these disease agents by means of diverse parts of their bodies (hairs body in addition to appendages and mouth parts) and secretions, which include regurgitate and faeces. Pathogenic microorganisms are pulled out up by flies from waste and other sources of refuse dumps, and then transferred on their mouth and other body parts through their vomits, faeces and contaminated external body parts to human and animal food (Babak *et al*., 2008 and Vasan, 2008). House flies cause mechanical transmission of pathogens, which is the most generally recognized mechanism (Sarwar, 2015; Pava-Ripoll, 2015 and Fisher *et al*, 2017). Nazari *et al.* (2017) stated that house flies (*Musca domestica*) have been known as a mechanical vector in transmit infectious diseases such as Cholera, Shigellosis, Salmonellosis and skin infections. Macovei and Zurek (2006) have reported that houseflies are important in food-handling and serving facilities habour and may have the capacity to transfer antibiotic-resistant and potentially virulent strains of pathogenic microorganisms. *Musca domestica* is capable of carrying

a variety of bacteria, viruses, fungi and parasitic diseases over its body appendages and can therefore pose a threat to the societal health, but despite the knowledge of the dangers posed by houseflies, the inability to keep a good sanitation leads, improper handling of food, random refuse dumping and little or no care of toilet facilities have led to an increase in the population of houseflies. The specificity of the pathogens carried by house flies depend on the area where the fly is collected (Zurek *et al*., 2014). The total numbers of bacteria isolated from flies trapped in the animal farms were higher than those isolated from flies caught in any other studied sites. Lower prevalence of the bacterial pathogens was isolated from flies caught in areas where sanitary conditions prevailed (Nmorsi *et al*., 2007). This study was designed to identify and determine the percentage of pathogens transmit by flies. To achieve these aims many parameters were used which is isolation of pathogens by culturing on different primary and specific media and identification of the isolates by colonies' characteristic features, gram staining and biochemical tests.

Materials and Methods

Collection of samples

A total of 60 flies were collected randomly, from different places in the animal farm. The method of collection was by the use of an electric flyswatter to capture the flies alive, and then the flies were killed by placed it in sterile universal container, properly labeled and transported immediately to the laboratory. In current study the flies' contamination were divided into two sections, one named S (surface) section, which means surface contamination with the flora. The other was named I (internal) section, which means internal contamination with flora. To prepare the S section each fly placed in a test tube containing 1.0 ml sterile normal saline by using a pair of forceps, the flies were gently rinsed by stirring with a glass rod in order to wash the microbial flora on the external parts of the houseflies into the normal saline then a drop of the normal saline from each tube was inoculated on nutrient and blood agar plates by streaking with the use of a flame sterilized inoculating loop, this was done in duplicates and around the flame to maintain aseptic condition. Nutrient broth was used to activate the isolates.

To prepare the I section the houseflies were collected from the test tubes and washed in ethyl alcohol to decontaminate their surfaces. Then it washed in normal saline to wash off excess alcohol that may affect the internal microbial flora during dissection. The flies were then each placed in sterile test tubes. The whole flies including guts and internal organ which placed in test tubes containing 1.0 ml of normal saline were homogenized individually. The resulting mixture was cultured and incubated in the same way as the external body surface.

Then inoculated plates were incubated in an aerobic condition at 37°C for 24 hours. After 24 hours of incubation, distinct colonies were selected randomly and sub-cultured on blood and nutrient agar to obtain pure cultures.

Cultural media

To cultivate the samples many different cultural media were used including blood agar, MacConkey, Nutrient agar, Eosin methylene blue, Mannitol salt agar and SS agar and incubated at 37°C for 48 hrs.

Biochemical tests

Many different biochemical tests were done to identify the bacterial isolates including Simmons citrate, TSI**,** Indole, catalase test, oxidase test, coagulase test IMVIC test and motility test (Quinn *et al*., 2004).

Results and Discussion

The results gave different percentage according to the isolation.

Result of the isolation showed that in case of surface contaminant 28 cases out of 60 samples were positive for *E. coli* (46.6%) and 4 cases were positive for *Klebsiella* out of 60 cases (6.6%) and 4 cases were positive for salmonella (6.6%) and 2 cases were positive

Table 1 : Results of bacterial isolation of pathogens from the flies.

No. of flies	No. of samples		Pathogens	No. of positive	Percentages
60	60	Surface flora	*E.coli*	28	46.6%
			Klebsiella	4	6.6%
			Salmonella	4	6.6%
			Pseudomonas	2	3.3%
			Streptococcus	30	50%
			Staphylococcus	12	20%
	60	Internalflora	*E.coli*	40	66.6%
			Salmonella	3	5%
			Proteus	4	6.6%
			Klebsiella	15	15%
	120				

Fig. 1 : MacConkey Agar showed pink colonies and yellowish colonies.

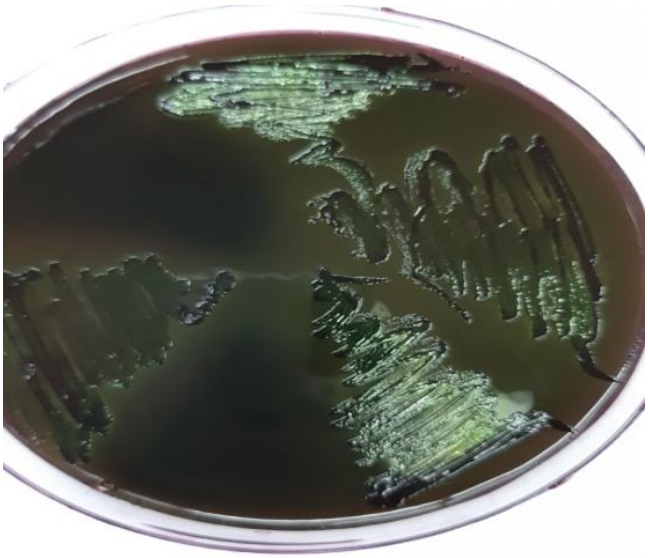

Fig. 2 : EMB Agar showed Metalic sheen color for *E.coli*.

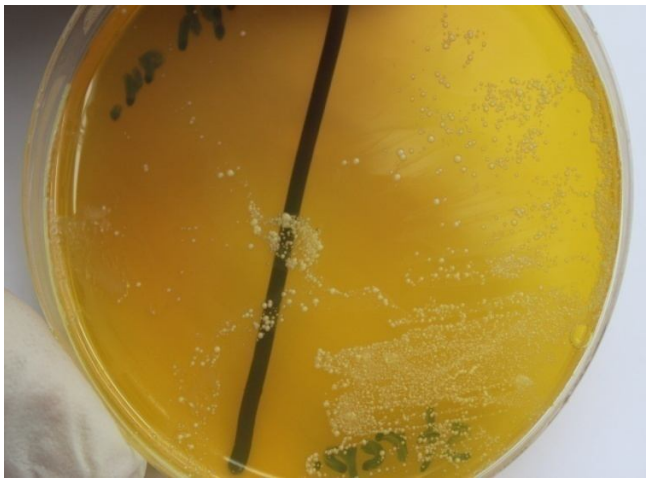

Fig. 3 : Mannitol salt Agar showed culture of staphylococcus.

Fig. 4 : MacConkey Agar showed culture of *Klebsiella*.

for *Pseudomonas* out of 60 (3.3%) and 30 cases were positive for *Streptococcus* (50%) and 12 were positive for *Staphylococcus* (20%). Also the results show that in case of internal contaminant 40 cases from 60 samples were positive for *E. coli* (66.6%) and 3 cases were positive for *Salmonella* (5%) and 4 cases were positive for *Proteus* (6.6%) and 15 cases were positive for *Klebsiella* (15%) (table 1 and figs. 1-4).

Our result agrees with Ibrahim *et al*. (2018) when he isolated many types of gram positive and gram negative bacteria from the flies. He demonstrates that *E.coli, Salmonella* and *Klebsiella* in addition to *Staphylococcus* and *Streptococcus* were transmitting by flies and he isolates these bacteria with different percentage. In this study the *E. coli* was the predominant isolates from the internal organ followed by *Klebsiella* and *Proteus* then *Salmonella* and this agree with Mawak and Olukose (2008), when he isolated the same spp of bacteria in addition to *Shigella* spp with close percentages. Also current study agree with Ahmed *et al*. (2018), who stated that *Pseudomonas*, *Staphylococcus* and *E. coli* were the most frequent bacteria isolated from houseflies. The results in this study agree with Vazirianzadeh *et al.* (2008) and Moosa-Kazemi *et al*. (2010), who declared the presence of *Pseudomonas*, *Proteus*, *E.coli* and *Klebsiella* on the external surface of house fly also agree with their mentioned that *Pseudomonas*, *Staphylococcus* and *E.coli* were the most frequent isolated bacteria while

the least present bacteria were *Klebsiella* and *Salmonella*.

In conclusion, the flies still have a big role in transmission of the microorganism which leads to dangerous diseases in addition to that animal's farms considered a good place for flies growth and maintenance, so the shortage in its observation and obligate usage of chemical substances to minimized the life cycle of flies will sophisticate the health problem and without the use of proper sanitation methods, flies will continue to replicate and disperse from adjacent areas. For that reasons, recommendations such as further studies to minimize the role flies in transmit the diseases to man and animals and usage the safe insecticide and sanitation of the farms to minimize flies effect is necessary.

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