

Isolation and enumeration of bacteria responsible for nosocomial infections from houseflies and determining their susceptibility to poison bait

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Abstract. Haeidari A, Keshavarzi D, Owlia P, Vatandoost H, Rafinejad A, Rafinejad J. 2021. Isolation and enumeration of bacteria responsible for nosocomial infections from houseflies and determining their susceptibility to poison bait. *Nusantara Bioscience* 13: 24-28. Nosocomial infections represent a serious public health concern in developing countries. Houseflies are one of the most common household pests carrying different pathogenic organisms. The purpose of this study was to isolate and enumerate bacteria species from house flies and to determine their susceptibility to Agita® fly bait. Flies were collected from two hospital environments between July to December 2014, in Yazd Province of Iran. Bacterial species were isolated from the outer surfaces of flies, and Agita® efficacy was evaluated based on lethal time (LT50) after 1, 2, 4, 8, 16, and 32 minutes. Three species of bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*) responsible for nosocomial infections have been isolated and enumerated from flies. Among the 30 flies collected, 17, 24, and 3 flies were contaminated with *E. coli*, *S. aureus*, and *Ps. aeruginosa*, respectively. The minimum number of isolated bacteria was 3×10^2 CFU/mL, whereas the highest number was 2.4×10^5 CFU/mL. The susceptibility results showed that, despite the existence of a significant effect for heterogeneity in both field and laboratory strains (p -value $< 0/05$) and an increase in the mortality of houseflies during the time, there was no significant difference between two strains regarding the efficacy of Agita® against houseflies. The findings of the present study revealed and confirmed that houseflies have an important role in the spread of nosocomial infections in hospital environments, and they are susceptible to Agita® fly bait.

Keywords: Agita, housefly, nosocomial infections, susceptibility

INTRODUCTION

The housefly (*Musca domestica*) is a common insect of the family Muscidae, order Diptera (Rassi et al. 2020). They are diurnal insects that live in the human environment and its surrounding, in terms of behavior, feeding, flying power, and quick handling, transmit mechanically many different pathogens to human's food and living environment (Zahn and Gerry 2020; Elyasigomari et al. 2020). There are more than 100 species that can transmit pathogens, but the two most common household pests are flies and cockroaches that transmit mechanically by oral supplements, hairs of body, legs, wings, feces, and vomit (Shiravand et al. 2018; Kobayashi et al. 2020). House flies and cockroaches can transmit various pathogens, including bacteria, protozoa viruses, and parasitic eggs that can cause internal disorders, diarrhea, and typhoid (Sahi 2019; Nwankwo et al. 2020). The hospital setting is one of the places that flies can cause serious problems for human health. Contamination in hospitals and medical centers to bacterial pathogenic agents (nosocomial infections), is the most complicated problem worldwide in both developed and developing countries (Nazari et al. 2017; Park et al.

2019). A previous study by Shiravand et al. (2018) indicated that nosocomial infection is a leading cause of death in all countries. Nosocomial infections have a significant impact on the length of hospital stay, mortality in hospitalized patients and medical care cost. *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* are the most important pathogenic bacteria causing nosocomial infections. The increasing antibiotic multidrug resistance of these bacteria has created many problems (Nwankwo et al. 2020). Therefore, the study of the number of pathogens carried by house fly is necessary to be done.

Chemical control is one of the most frequently used interventions to control house flies (Levchenko et al. 2019). Poison bait formulations such as Agita are one of the current control strategies, as it is cost-effective and reduces insecticide exposure to non-target organisms. Agita bait is a contact and stomach insecticide with a mixture of two active ingredients; thiamethoxam (a neonicotinoid insecticide) and tricosene (a housefly pheromone) (Nurita et al. 2008; Ong et al. 2015). The toxicity mechanism of the neonicotinoids is an agonist to the nicotinic acetylcholine receptor. Tricosene is used in this formulation to attract

male and female flies (Nurita et al. 2008). There is no information on the house fly susceptibility to the Agita fly bait in Iran. Therefore, the objective of this study was to determine the efficacy of house fly bait formulations against *M. domestica* under laboratory conditions.

MATERIAL AND METHODS:

Study area and sampling

Two public hospitals were randomly selected from two areas of Yazd between June to December 2014 for this descriptive-analytic study. There were 30 adult houseflies trapped from various parts of the hospitals. Collected houseflies were put into sterile test tubes and then transported to the microbiology laboratory within two hours. Yazd is the driest city in Iran, with a yearly precipitation amount of 49 millimeters (1.9 in) and only 23 days of precipitation, with summer temperatures very frequently above 40°C (104°F) in blazing sunshine without humidity.

Microbiological analysis

Each fly was placed in 5 mL physiologic serum and was vortexed for 30 seconds. Then, 100 µl of physiologic serum was inoculated in the Brain Heart Infusion Agar culture medium. Inoculated Petri dishes were incubated for 24 hours at 37°C. After incubation, the appearance of the colonies was observed, and the number of colonies was counted.

One hundred µl physiologic serum was culture on the various culture media, i.e., Nutrient Agar, Brain Heart Infusion Agar, Cetrinide Agar, Brad Parker Agar (BPA) and MacConkey Agar (production of IBRESCO Company, Iran) and Mannitol Salt Agar (MSA) and Eosin Methylene Blue (EMB) (production of MERCK Company, Germany) to specify cultivation of *P. aeruginosa*, *E. coli*, and *S. aureus*. The various inoculated culture media were incubated for 24 hours at 37°C. After incubation, the number of colonies based on the specific characteristics of the bacteria was determined.

Chemicals and Preparation

Agita® 10 WG (Basel, Switzerland) contains thiamethoxam (10.0% w/w) and Tricosene (0.5g/kg). The required concentration was prepared according to the manufacturer's instruction (10g/13mL water) and was rubbed onto three paper sheets (10 cm x 10 cm) as bait targets and placed in separate cages. Three replicates were conducted for each bait test (20 flies for each replicate). In the control group, the paper was soaked with water.

Bait evaluation

The first generation (F1) of adult flies were used for testing. Flies were starved for 12 h before the tests. Efficacy was evaluated based on lethal time (LT50) after 1, 2, 4, 8, 16 and 32 minutes of contact time. After contact times, flies were transferred into a disposable cup and the mortality was scored 24 hours later. The mortality of the flies was assessed daily at a certain time for 2 weeks. Data

were analyzed using Probit analysis. The 95% confidence interval overlap of LT50 was compared for statistical significance.

RESULTS AND DISCUSSION

Isolation and enumeration of bacteria species

Thirty flies were used to isolate the bacteria responsible for nosocomial infections. There were 25 flies (83.3%) were found to carry one or more species of bacteria. Seventeen flies were carrying *E. coli*, 24 flies were carrying *S. aureus*, and 3 flies were contaminated with *P. aeruginosa* respectively. There were 2 flies (6.7%) carrying 3 bacteria species, and 16 flies (53.4%) carrying *E. coli*, and *S. aureus* species. *E. coli* contamination was observed by the appearance of metallic luster colony from 17 flies samples (Figure 1). The minimum number of isolated bacteria was found to be 3×10^2 CFU/mL, while the highest number was 2.4×10^5 CFU/mL (Table 1.). The housefly (*Musca domestica*) has a wide distribution in all parts of the world and a close association with humans and their environment, therefore housefly can be one of the most important mechanical vectors of bacteria, viruses, fungi, protozoa, and worm eggs (Zahn and Gerry 2020; Elyasigomari et al. 2020). The results of bacterial isolation from the outer surface of the fly body show that flies can be carriers of various species of bacteria and vector of several diseases because they are common around households, garbage, human, and animal excreta (Pace et al. 2017; Kavran et al. 2019). This study showed that house flies can carry dangerous bacteria such as *P. aeruginosa*, *E. coli*, and *S. aureus* that are medically important. *P. aeruginosa* is associated with a progressive loss of lung function in cystic fibrosis patients (Mogayzel et al. 2014).

Staphylococcus aureus contamination in houseflies has been reported in several previous studies (Nazari et al. 2017; Nwankwo et al. 2020). One of the most common human pathogens and the most important cause of hospital-acquired infections is *S. aureus*. *S. aureus* can cause a wide range of diseases, including serious infections such as septicemia, endocarditis, wound infections, bacteremia, and osteomyelitis in hospitalized patients and is one of the common causes of mortality in hemodialysis patients (Salgado-Pabón et al. 2013; Tong et al. 2015).

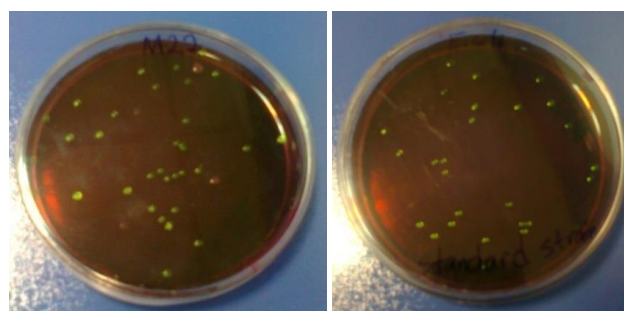


Figure 1. Green metallic luster colonies belong to the standard strains of *Escherichia coli* (right) and M22 fly (left) on the EMB growth medium

Table 1. Total bacteria count and bacteria species isolated from the body surfaces of the collected flies

Sample No.	Count			Sample No.	Count		
	<i>E. coli</i>	<i>S. aureus</i>	<i>Ps. aeruginosa</i>		<i>E. coli</i>	<i>S. aureus</i>	<i>Ps. aeruginosa</i>
M1	9×10^2	10^2	---	M16	---	9×10^2	---
M2	4×10^2	2×10^2	---	M17	10^2	3×10^2	---
M3	8×10^3	6×10^3	---	M18	---	6×10^2	---
M4	2×10^2	10^2	---	M19	6×10^2	4×10^2	---
M5	3×10^3	10^2	---	M20	---	---	---
M6	5×10^3	3×10^3	3×10^2	M21	---	---	---
M7	4×10^3	4×10^2	---	M22	2×10^3	---	---
M8	8×10^2	1.1×10^4	---	M23	---	10^2	---
M9	1.6×10^3	2×10^3	---	M24	---	3.4×10^3	---
M10	4.6×10^3	7×10^3	---	M25	---	3.8×10^2	---
M11	---	---	---	M26	---	---	---
M12	---	---	---	M27	10^2	4.3×10^3	---
M13	9×10^2	5×10^2	---	M28	---	1.9×10^2	---
M14	5×10^2	4×10^2	---	M29	---	2×10^2	---
M15	8×10^2	8×10^2	4×10^2	M30	---	1.2×10^2	---

Table 2. Probit regression line parameters related to the results of experiments of Ajita insecticide on two field and laboratory strains.

Tests	Y-intercept	Slope \pm SE	LT ₅₀ , 95% C.I.	LT ₉₀ , 95% C.I.	X ² (df)	p value
Field strain	-1/41	1/92 \pm 338	5/47	25/47	45/3 (4)	</05
Lab strain	1/48	1/77 \pm 237	6/85	36/42	24/3 (4)	</05

Escherichia coli contamination had the highest frequency of occurrence which is in line with previous studies (Salgado-Pabón et al. 2013; Nwankwo et al. 2020; Sobur et al. 2019). *E. coli* and *S. aureus* are the two most important pathogens causing urinary tract infections in humans (Zahn and Gerry 2020). A study by Chaiwong et al. (2014) in Thailand showed that bacterial contamination (*P. aeruginosa*, *E. coli*, and *S. aureus*) in *C. megacephala* was 11-12 times greater than *M. domestica*.

Sasaki et al. (2000) reported a similar result on *E. coli* contamination in house flies. *E. coli* reproduce in the oral parts of the housefly, and three days after swallowing the bacteria was disposed of by the fly, and survive for up to four days in the gut of the housefly. House flies transmit bacteria both mechanically, via contaminated mouthparts and legs, and biologically, via ingestion of microbes and excretion in vomit or feces. (Sasaki et al. 2000). We assume that the bacteria are multiply in the digestive tract of houseflies. The digestive tract of house fly may provide a favorable environment for the horizontal transfer of antibiotic resistance genes among bacteria (Zahn and Gerry 2020). Therefore, flies may be involved in the spread of drug-resistant bacteria in different places.

In the present study, bacterial contamination in houseflies was very high including *E. coli*, *Ps. aeruginosa*, and *S. aureus*. These bacterial isolates were medically very important because they can result in serious infections. These bacteria were collected from the outer surface of the housefly's body, therefore, bacterial contamination on

houseflies collected from the hospital environment may reflect the contamination status of the hospital environment.

Bait evaluation

The mortality rate showed that the death occurred immediately after the flies feed the feeding from Agita. One minute after feeding, there were 22 flies from the laboratory and 26 flies from the field were dead. Over time, the mortality rate of flies in both strains increased (Figure 2).

The results of probit analysis of the susceptibility tests revealed that with increasing time (after one minute), the probability of death of house fly collected from the field was increased to 1.928 ± 0.38 , while that of the house fly from the laboratory was 1.77 ± 0.128 . The median lethal time (LT₅₀) and LT₉₀ for houseflies from the field were 5.47 minutes and 25.47 minutes, respectively. LT₅₀ and LT₉₀ values for houseflies from the laboratory were 6.85 and 36.42 minutes, respectively (Table 2.). The regression lines of the susceptibility for houseflies from the field and laboratory were presented in Figure 3. The susceptibility results showed that, despite the existence of a significant effect for heterogeneity in both species of field and laboratory (p-value < 0/05) and an increase in the mortality of houseflies during the time, there was no significant difference between two strains regarding the efficacy of Agita® against houseflies.

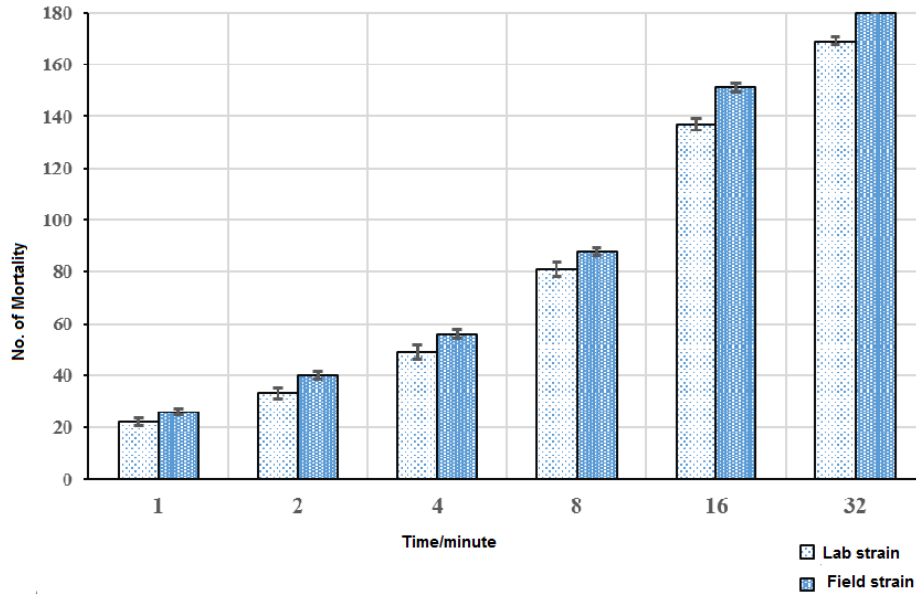


Figure 2. Effect of Agita on the number of mortality in adult flies during specific times.

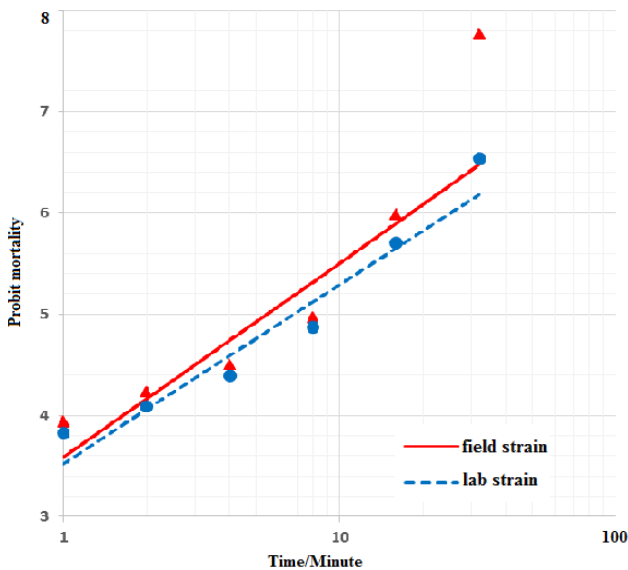


Figure 3. Probit regression lines of susceptibility test for adult flies to Agita fly bait

The results of the susceptibility tests showed that the resistance ratio was 0.79. Resistance ratio (RR) was obtained according to the following formula :

$$RR = \text{LT50 of field population} / \text{LT50 of susceptible laboratory population}$$

Based on the RR value, houseflies collected from the field were more susceptible to Agita. A previous study by Nurita et al. (2008) on synanthropic flies showed that the Agita fly bait was effective for 4 weeks but its effectiveness was significantly reduced on week 6th. A

study by Ong et al. (2015) indicated a minor resistance to Agita, hence there is a possibility of increasing Agita resistance in houseflies. Agita irritates before the knockdown effect and consequently, the houseflies fly away and die away from the insecticide source (Msangi et al. 2005). These results indicate that Agita fly bait performs well against houseflies. Evaluation of the residual efficacy of insecticides or chemical baits is important in formulating effective fly control programs. There is a need to extend the effectiveness of fly baits for a longer period and economically feasible to use in fly control programs (Nurita et al. 2008). It has been reported that the effectiveness of Quick Bayt® is longer compared to Agita®. The difference in effectiveness could be due to pellets or granules of Quick Bayt® are homogenous pellets (Nurita et al. 2008).

The results of this study are expected to motivate the relevant hospital authorities regarding insect control so that it can prevent infections caused by bacteria and stem hotspots of their release in the hospitals. Agita fly bait can be used as part of an integrated pest management program for house flies and also for application as a spot spray insecticidal bait. The presence of houseflies indicates a lack of sanitation and unhygienic conditions. The findings of this study suggest that houseflies can be efficient vectors for the mechanical transmission of multidrug-resistant diseases causing organisms, especially from a health-care environment. Considering the importance of this issue: hospital officials should pay attention to healthcare waste management, hospital inputs particularly windows covered with a net to prevent the entry of flies, if necessary. The Agita fly bait formulation can be used for outdoor fly control in the hospital.

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