

Identification of bacteria and parasites with medical importance present in common fly (*Musca domestica*), captured in a highly marginated community

Identificación de bacterias y parásitos de importancia médica presentes en mosca común (*Musca domestica*), capturadas en una comunidad de alta marginación.

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Abstract

The common fly (*Musca domestica* L.) is a mechanical vector and can be a biological vector through ingestion and regurgitation. In rural communities is common to observe numerous populations of this, consequence of multiple unsanitary sites. The present study was carried out in order to identify the species of bacteria and parasites with medical importance present in the common fly, captured in homes and microhabitats (chicken coops, pigsty and backyards) in homes of the community of Cholul, Cantamayec. 20 species of bacteria were identified: *E. coli* (41.78%) was the most frequent species in all processed flies. In turn, households (45.20%) were the place where the greatest amount of bacterial species was isolated, being *E. coli* (42.42%), the frequent species, in the same way in pigsty (45.65%), chicken coops (38.09%) and backyards (30.76%). Regarding the enteroparasites identified from the digestive cavity of flies, *Endolimax nana* (38.46%) was the frequent cyst and *Ascaris lumbricoides* (15.38%), the only nematode found. In turn, the pigsty (n = 5) was the site with the highest number of isolated enteroparasites. The results obtained show that flies carry pathogens that could be involved in human infections of community origin.

Musca domestica, Enteroparasites, Microhabitats

Resumen

La mosca común (*Musca domestica* L.) es un vector mecánico, puede ser vector biológico mediante la ingestión y regurgitación. En las comunidades rurales es común observar numerosas poblaciones de ésta; consecuencia de múltiples sitios insalubres. El presente estudio se llevó a cabo con la finalidad de identificar las especies de bacterias y parásitos de importancia médica presentes en la mosca común, capturadas en casas y microhábitats (gallineros, chiqueros y patios) en domicilios de la comunidad de Cholul, Cantamayec. Se identificaron 20 especies de bacterias, la cual: *E. coli* (41.78%) la especie frecuente en todas las moscas procesadas. Las casas (45.20 %), resultó el sitio donde se aisló mayor cantidad de especies bacterianas, siendo *E. coli* (42.42 %), la especie frecuente, de igual modo en chiqueros (45.65 %), gallineros (38.09 %) y patios (30.76 %). En cuanto a los enteroparasitos identificados de la cavidad digestiva de las moscas, *Endolimax nana* (38.46 %) fue el quiste frecuente y *Ascaris lumbricoides* (15.38 %) el único nematodo encontrado. A su vez el chiquero (n = 5) fue el sitio con mayor cantidad de enteroparasitos aislados. Los resultados obtenidos demuestran que las moscas portan patógenos que podrían estar involucrados en infecciones humanas de origen comunitario.

Musca domestica, Enteroparásitos, Microhábitats

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Introduction

For the population, a large number of arthropod species are important in the medical field, mainly insects, since they act as vectors, or carriers of microorganisms that cause infectious diseases, transmission is carried out in two ways: mechanical transmission (by drag mechanical transmission of infectious or parasitic agents such as flies or cockroaches) and biological transmission (such as bites or defecation) (Calderón (2004)

According to the World Health Organization (WHO), vector-borne diseases represent at least 17% of infectious diseases, and cause approximately 700,000 deaths each year, the distribution of these diseases is determined by demographic, environmental and social factors and is they occur more frequently in tropical areas and developing countries, mainly affecting the population of low socioeconomic level (Cabrera M, Verástegui M, 2005).

In numerous studies, the presence of infectious agents such as *Escherichia* has been shown in the common fly. Col, i *Salmonella typhi*, *Shigella flexneri* among others; and its role as a mechanical vector (Béjar C et al., 2006; Brazil et al., 2007; Quiceno et al., 2010). However, one of the most important biological vectors can also be one of the most important due to the protective effect that the pathogen gives inside its body, representing another of the potential pathways through ingestion, regurgitation prior to each meal and defecation of the pathogens. The alternation of habitat during the day makes it a dangerous vector when moving in the kitchens and dining rooms of homes. (Crosskey & Lane, 1993; Fernández B. et al., 2014)

Among the main infectious diseases transmitted by *M. domestica* are those that come from food, water contamination, fomites and poor sanitation, causing gastrointestinal infections, mainly those that produce diarrhea, the most important cause of morbidity and mortality worldwide (Castillo et al, 2012). These diseases are the leading cause of mortality in the age group under 4 years of age, whose rate is estimated at 3.3 million per year for Latin America, Africa and Asia; They are considered one of the main factors that contribute to child malnutrition and hospitalization (Cárdenas & Martínez, 2004).

In Mexico during 2019, among the main causes of disease, intestinal infections still remain of epidemiological importance. In the state of Yucatán, salmonellosis and intestinal infectious diseases by other organisms occupy the first places (Secretary of Health & General Directorate of Epidemiology, 2019). In Yucatán, the population most vulnerable to suffering from acute diarrheal disease are children under 5 years of age, since in the state, due to the habits and customs ingrained in the population, hygiene practices are almost nil, which produces the increase of these diseases (Peña 2012).

The agents involved in infectious diseases are mostly enteric bacteria of the Enterobacteriaceae group, because *M. domestica* mainly feed on feces and other waste that are a rich source of these pathogens, including enteropathogenic strains such as enteroaggregative *E. coli* (EAEC), Enterohememagical *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), and Enteropathogenic *E. coli* (EPEC), *Vibrio cholera*, and *Bacillus anthracis* that cause enteric disease, cholera, and anthrax, respectively, and others including: *Klebsiella* spp, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Clostridium* spp. and *Enterococcus*, to name just a few (Solá-Ginés et al., 2015). Also some species of parasites belonging to the genera: *Ascaris*, *Entamoeba*, *Trichiuris*, hookworms and protozoa cause enteric diseases with various clinical pictures, among which are: abdominal pain, nausea, anorexia, fatigue, diarrhea and weight loss (Cárdenas & Martínez, 2004; Eke et al., 2016)

In rural communities it is common to observe numerous populations of *M. domestica*, particularly in the vicinity of markets, food outlets, homes, garbage dumps, animal farms in general and educational facilities, with greater emphasis where sanitary management is inappropriate being these the main sources of origin of microbial infections. Animal farms represent an important source of the spread of *M. domestica*, nutrient-rich substrates such as animal manure provide excellent development and larval feeding of flies. (de Román et al., 2004; Gallego Berenguer, 2014)

In studies carried out to identify the suitability of the flies' growth sites in descending order, it was found that horse manure, human excrement, cow manure, fermentation of vegetable and kitchen residues are optimal sites for development. In the same way, pens for pigs, horses, sheep, cattle and poultry are places where flies concur, being pig pens where there are more flies, however, the others are not omitted as a possible source of spread. Likewise, fruits and vegetables, garbage piles, and compost are also highly favored sites for development, and these types of microhabitats are quite common in communities. (Sarwar 2016)

Despite the presence of abundant populations of *M. domestica*, coupled with the relatively high prevalence of parasites due to lack of hygiene, there is no knowledge of documented studies in the interior of the state of Yucatán, on the isolation of parasites in *M. domestica*. In view of the importance, this research was carried out in order to isolate and identify pathogens of medical importance in *M. domestica* from a rural community, as well as micro habitats to determine the possible sources of spread of pathogens. and the microbiological risks of environments, where the common fly occurs and constitutes the first study carried out in a rural community in the state and provides information for future research.

Materials and methods

Study site

The study was carried out in the community of: Cholul, Cantamayec, Yucatán located at the parallels: 20 ° 26'30.00'' N and 89 ° 09'11.00'' W. It has an average height of 24 meters above sea level. It is bordered by the following municipalities: to the north with Sotuta, to the south with Tixméhuac and Chacsinkín, to the east with Yaxcabá and to the west with Teabo and Mayapán. The region is classified as warm subhumid, with rains in summer (May - July), which when interrupted are the so-called mid-summer droughts, has an average annual temperature of 26.3 °C and an average annual rainfall of 1,200 millimeters. The prevailing winds come in an east-west direction. Average annual relative humidity, ranging from 66% to 89% (Tolrá Hjorth-Andersen, 2015).

Collection and identification of flies

15 homes were sampled, during the months of March and June 2018, the flies were captured in sites inside the house, patio, chicken coops and pens as a reference for the search for possible sources of contamination of the flies. A minimum of five flies were captured per site. For the collection, backpack backpacks (BKPr. México®) and entomological nets were used. The entomological analysis and taxonomic determination of *M. domestica* was carried out in the microbiology laboratory and supported by dichotomous keys (Murray et al., 2004)

Sample processing

The collected insects were stored in sterile plastic bottles with refrigerants and transferred to the microbiology laboratory, in a period of less than 24 hours. In a laminar flow hood, five flies were separated with sterile forceps into 1.5 mL conical microcentrifuge tubes and labeled with the sample code.

Sediment culture

External structure of the fly

To each tube with the five flies inside, 500 µL of sterile physiological saline was added and the supernatant with a previously sterilized and cooled round bacteriological loop was stirred for 1 min, the seeding was carried out by the cross-streak plate method. turning the box while scratching to form a pentagon in the agar: MacConkey (BD Bioxon, Becton Dickinson. México®), selective medium for Enterobacteriaceae and gram negative bacilli, salty Mannitol agar (BD Bioxon, Becton Dickinson. México®), medium selective for staphylococci and gram positive bacteria. At the end, they were placed in a bacteriological oven (Riossa series: ECML. México®), at 37 ° C for 24 hours.

Internal structure of the fly

With sterile entomological forceps, the flies were placed in a 1.5 mL conical microcentrifuge tube, 500 µL of 70% alcohol was added, mixed by inversion, and the supernatant was discarded. This step was repeated three times. To remove excess alcohol, it was washed with 500 µL of sterile physiological saline and stirred by inversion.

At the end of the washes with sterile forceps, the flies were placed on object slides, with number 11 scalpels (DLP, Dentilab. México), A sagittal cut was made in the abdomen to extract the digestive cavity, it was passed in a conical microcentrifuge tube and 500 µL of sterile physiological saline was added to macerate with sterile pistils until the sample was homogenized. Subsequently, the sowing was carried out by the cross-streak method on a plate, turning the box until a pentagon was formed in the agar: MacConkey (BD Bioxon, Becton Dickinson. México®) and salty Mannitol (BD Bioxon, Becton Dickinson. México®), to take them to a bacteriological oven (Riossa series: ECML. México®), at 37 ° C for 24 hours.

Observation of parasitic forms

To observe the presence of protozoa and nematodes, 100 µL of saturated saline solution was added to each conical tube of the external wash and internal maceration, then 10 µL of each sample was taken to pass to a slide in which a drop of Lugol was added. (HYCEL. México®), to observe them at 10x and 40x in an optical microscope.

Identification of bacteria and parasitic forms

Tables from the Manual Of Clinical Microbiology were used to identify pathogenic bacteria. 2013, 8th Edition of Murray. For the parasitic forms, tables from the book: Microbiology and Human Parasitology were used. 2007, 3rd Edition of Romero. (Murray et al., 2004; Romero, 2007)

Results

Specimens of *M. domestica* were collected in 15 homes in the town of Cholul, Cantamayec municipality, of which 140 belonged to homes, 95 to pens, 45 to chicken coops and 25 to yards. Of the total of specimens, 61 samples were processed, from which 146 strains of different species of bacteria were isolated.

The *Escherichia coli* species was the most frequent (41.78%), followed by coagulase negative *Staphylococcus* (12.32%), *Enterobacter* spp. (10.95%), *Klebsiella* spp. (5.47%) and *Providencia* spp. (5%), the following species were also isolated in less frequency: *Enterobacter sakasakii* and *Klebsiella pneumoniae* (2.73%). *E. agglomerans*, *Shigella* spp, *Staphylococcus aureus*, *Proteus* spp., *Citrobacter* spp. and *Serratia* spp. (2.05%). *Morganella* spp., *Klebsiella oxytoca* and *Edwardsiella* spp. (1.36%). *Morganella morgani*, *K. ozaenae*, *Shigella flexneri* and *Yersinia* spp. (0.68%). Specifically, table 1 shows in detail the number of the main species isolated by processed structure (external and internal) in *M. domestica*. The results demonstrate the presence of different bacterial species of medical importance in *M. domestica*. However, strains of *E. coli*, coagulase negative *Staphylococcus*, *Enterobacter* spp., *Klebsiella* spp. Were found more frequently and *Providencia* spp.

Species	External structure	Internal structure	Total
<i>Escherichia coli</i>	23	38	61
<i>Staphylococcus coagulasa negativo</i>	9	9	18
<i>Enterobacter</i> spp.	10	6	16
<i>Klebsiella</i> spp	2	6	8
<i>Providencia</i> spp.	4	3	7

Table 1 Number of most frequent bacterial species isolated, by processed structure in *M. domestica*

Regarding the capture sites, frequency and species of isolated bacteria, differences were obtained in all the capture sites, in (Table 2), it can be observed that the houses (45.20%), was the site where the highest isolation was number of bacteria, and the most frequent species were: *E. coli* (42.42%), *Staphylococcus coagulase negativo* (15.15%), *Enterobacter* spp. (10.60%) and *Klebsiella* spp. (7.57%).

Species	Houses	Pigsty	Hen house	Courtyard
<i>Escherichia coli</i>	28	21	8	4
<i>Staphylococcus coagulasa negativa</i>	10	6	0	2
<i>Enterobacter</i> spp.	7	0	6	3
<i>Klebsiella</i> spp.	5	2	0	1
<i>Providencia</i> spp.	3	4	0	0
<i>Enterobacter sakasakii</i>	1	1	1	1

Table 2 Number of the most frequent bacteria isolated, by capture sites

On the other hand, the pigpen was the second site where the highest frequency of bacteria was isolated (31.50%), in the same way *E. coli* was the most isolated species (45.65%), followed by *Staphylococcus coagulase negative* (13.04%) and *Providencia* spp. (8.69%). In the same way, 14.38% of the total isolated species were isolated in chicken coops, with *E. coli* the most frequent species (38.09%), followed by *Enterobacter* spp. (28.57%) and *Serratia* spp. (14.28%). Finally, 8.09% of the total isolated species were isolated in patios, as in the other sites, *E. coli* was the most frequent species (30.76%), followed by *Enterobacter* spp. (23.07%) and *Staphylococcus coagulase negative* (15.38%). (Table 2).

An interesting pattern analyzed in this study revealed that *Staphylococcus coagulase negative* was the second species isolated in: houses (15.15%), pens (13.04%) and yards (15.38%), which confirms its wide distribution in nature and its clinical significance. In most cases it is difficult to establish since it can be commensal found in the microbiota of the skin and mucous membranes of mammals including man and birds. Table 3 shows in detail the species and number of bacteria isolated by structure processed in *M. domestica* in the different capture sites and the number of strains isolated in the internal and external parts of the fly, being *E. coli*, the most commonly found species.

Especies	Houses		Pigsty		Hen house		Courtyard	
	External	Internal	External	Internal	External	Internal	External	Internal
<i>Escherichia coli</i>	10	18	8	13	3	5	1	3
<i>Staphylococcus coagulase negativa</i>	5	5	3	3	0	0	1	1
<i>Enterobacter</i> spp	3	4	0	0	3	3	2	1
<i>Klebsiella</i> spp	1	4	0	2	0	0	1	0
<i>Providencia</i> spp.	2	1	2	2	0	0	0	0
<i>Enterobacter sakazakii</i>	1	0	0	1	0	0	0	1
<i>E. agglomerans</i>	0	1	0	0	1	1	0	0
<i>Klebsiella pneumoniae</i>	1	2	0	1	0	0	0	0

Table 3 Number of isolated strains per bacterial species in internal and external structure processed of *M. domestica* in each capture site

Similarly, the presence of *Enterobacter* spp. in chicken coops (28.57%), patios (23.07%) and houses (10.60%) it is highly relevant for this study. The medical importance lies particularly in: *Enterobacter aerogenes* and *E. cloacae* since they have been associated with nosocomial outbreaks and are considered opportunistic pathogens. *Enterobacter* spp. can cause numerous infections such as: brain abscess, pneumonia, meningitis, septicemia, urinary tract (especially related to catheter) and abdominal cavity and intestinal infections.

Regarding the parasites isolated in *M. domestica*, the results shown were the following: 13 enteroparasites were identified in the internal macerates of *M. domestica*, of which four cysts were isolated (Figure 1A and 1B), *Endolimax nana* being the most frequent (38.46%), followed by *Entamoeba* spp. (30.76%), finally *Blastocystis* spp, and *Cryptosporidium* spp. (7.69%). A nematode egg belonging to *Ascaris lumbricoides* (15.38%) was also identified (Figure 1C). The pigsty was the site where the highest number of enteroparasites was found (n = 5), in Table 4, you can see the parasites isolated from the internal macerates of *M. domestica* in the four sites collected.

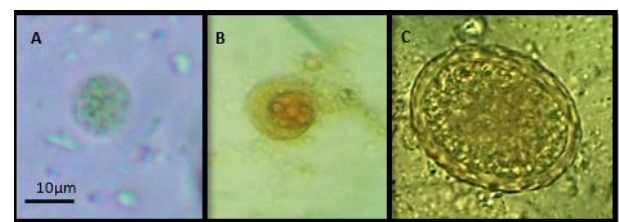


Figure 1 Lugol staining in saturated saline solution, parasites observed under light microscope at 40x. (A) *Endolimax nana* cyst. (B) cyst of *Entamoeba* spp. (C) egg of *Ascaris lumbricoides*

Parasites	Hen house	Pigsty	Courtyard	Home	%
<i>Entamoeba</i> spp.	0	0	2	2	30.76
<i>Blastocystis</i> spp.	1	0	0	0	7.69
<i>Endolimax nana</i>	2	3	0	0	38.46
<i>Cryptosporidium</i> spp.	0	0	0	1	7.69
<i>Ascaris Lumbricoides</i>	0	2	0	0	15.38
Total (n):	3	5	2	3	100

Table 4 Parasites identified in internal macerates of *M. domestica*

Discussion

Currently there are no studies that report the frequency of Enterobacteriaceae in flies from pig pens and poultry houses in rural areas, the objectives are focused on the isolation of bacteria resistant to various antibiotics in pig and poultry farms, which makes sense for its commercial value. However, these types of studies provide an overview of the frequency of bacteria isolated from these sites compared to bacteria isolated from poultry houses and pens in rural areas.

The high percentage of bacteria isolated inside houses represents a greater concern, compared to pens, chicken coops and yards, which are optimal sites for the reproduction, propagation and dissemination of a greater quantity of pathogenic bacteria, due to the manure generated and organic matter in decomposition of the place. However, the sanitary conditions in the houses, the lack of protection in windows and doors against the entry of flies from unhealthy places with abundant fecal matter, such as chicken coops and pens, favor the synanthropic behavior of *M. domestica*. The mentioned characteristics of the environment and the conditions of the house as a whole are factors that could increase the frequency of *E. coli* in flies due to their ability to fly. However, the food that is prepared inside the houses could be the factor that favors the attraction of a greater number of flies and with it the high percentage of isolates of *E. coli* in these places.

Although the number of species isolated in patios was less, the results obtained reflect the sanitary conditions of the same in the community. *E. coli* is found in the intestines of animals and humans, it is attributed an important role in gastrointestinal and urinary infections. This characteristic is verified in the present study by analyzing a greater number of *E. coli* strains in the internal macerates of *M. domestica* at the capture sites. Which due to the regurgitation of the fly and the manure generated by the backyard animals could be the cause of the frequency of this pathogen, however, it was also isolated in the external part which would represent a risk of transmission.

The results agree with the study carried out by Cervelin et al. (Cervelin et al., 2018), where they isolated enterobacteria associated with *M. domestica* as an indicator of infection risk in pig production farms, in their results they found a high frequency of *E. coli* in flies (104 to 106 CFU per 20 flies). This finding was directly correlated with the high concentration of fecal matter that was in the place, this characteristic was the same observed in the community pens, the lack of cleanliness of the pens, are factors that favor the conditions for the development of lots of flies.

The presence of *Enterobacter* spp. in chicken coops (28.57%), yards (23.07%) and houses (10.60%), it is relevant, but currently there are no studies reporting the effect as a pathogenic agent of diseases and its possible transmission by flies, despite this, reports by Lamiaa et al., (2007), confirm its presence in flies from urban areas. For its part, the study by Nazni et al., (2005), was concentrated in yards, landfills, food processing areas and poultry farms, obtaining similar results.

Solá et al. (2012), analyzed the bacteria from flies in poultry farms, in their high frequency reports of *E. coli* (81%), it was attributed to the state of contamination of the farm, this characteristic observed in farms is similar to that observed in community; the difference is that the homes have little delimited chicken coops, this allows the birds to be in patios excreting throughout the area, even inside the houses; this fact increases the contamination of the place, which explains the frequency of *E. coli* in flies trapped in poultry houses. The foregoing supports that the characteristics of the environment contribute to the frequency of this species in *M. domestica*. The study carried out by Blaak et al., (2015), where they studied the distribution and diversity of *E. coli* in the environment of a poultry farm, confirms the contribution of these sites in the contamination of the environment and is consistent with the finding that it was detected in flies (15%).

The presence of *Serratia* spp. Although in a low percentage, in poultry houses it differs from that reported by Lamiaa et al (2007), in this study areas with the presence of backyard birds were included. Despite this, it is known to be an opportunistic pathogen and is one of the ten most common causes of bacteremia in North America. They are responsible for a variety of infections, including bacteremia, pneumonia, intravenous catheter-associated infections, osteomyelitis, endocarditis, and rarely, endogenous and exogenous endophthalmitis (Biedenbach et al., 2004; Van Houdt et al., 2007). Until recently, *Serratia* was considered to be a pathogen, mostly nosocomial, but one study (Laupland et al., 2008) showed that 65% of infections with *Serratia* species were actually of community origin. Which could confirm the presence in backyard chicken coops of the community.

The results obtained in this study are also similar to that reported by Muñoz & Rodríguez, (2015) where species of enteroparasites were found: *Blastocystis* spp. (35.1%), *Endolimax nana* (2.7%) and *Entamoeba* spp. (16.2%). Similarly, Guillén-Tantaleán et al (1984) analyzed a total of 900 flies captured in garbage dumps, homes and stables, in their results they found cysts of: *Endolimax nana* and *A. lumbricoides* eggs.

The problem of isolating *A. lumbricoides* in flies is the high frequency in infants in rural and urban communities, which is associated with developmental delay affecting the growth of infants, proof of this is the study carried out by Gutiérrez-Jiménez et al., (2019), where they observed a high prevalence of stunting in children from rural regions (79.8%), than urban ones (7.5%). And only children from rural municipalities were parasitized (72.6%), with *A. lumbricoides* and *Entamoeba histolytica* / *Entamoeba dispar* being the most prevalent parasites (57.1 and 38.1%, respectively).

Cárdenas & Martínez (2004), reported: *Cryptosporidium* spp., and *Endolimax nana* in internal macerates of *M. domestica* from garbage dumps and houses with poor sanitary conditions, according to the authors of this study, these findings were the causal agents of diseases in the population. *Cryptosporidium* spp. and *Blastocystis* spp. they are considered pathogens for man, they produce diverse clinical pictures, among which abdominal pain, nausea, anorexia, fatigue, diarrhea and weight loss stand out. The presence of them could be due to the insect's habit of living in contact and feeding on decomposing matter, mainly fecal from man and animals Van Houdt et.al (2007).

Conclusions

Flies are potential vectors of pathogens and are associated with the sanitary conditions of the environment where they develop, this behavior is corroborated with the results we obtained in the present study where 20 species of bacteria were identified: *Escherichia coli* (41.78%), *Staphylococcus coagulase negative* (12.32%), *Enterobacter* spp (10.95%), *Klebsiella* spp (5.47%), *Providencia* spp (5%), *Enterobacter sakasaki* and *Klebsiella pneumoniae* (2.73%). *Enterobacter agglomerans*, *Shigella* spp, *Staphylococcus aureus*, *Proteus* spp, *Citrobacter* spp and *Serratia* spp (2.05%).

Morganella spp, *Klebsiella oxytoca* and *Edwardsiella* spp (1.36%). *Morganella morgani*, *Klebsiella ozaenae*, *Shigella flexneri* and *Yersinia* spp (0.68%); four protozoan cysts: *Endolimax nana* (38.46%), *Entamoeba* spp (30.76%), *Blastocystis* spp (7.69%) and *Cryptosporidium* spp (7.69%); a nematode egg: *Ascaris lumbricoides* (15.38%). In the case of bacteria, *E. coli* (41.78%) was the most frequent isolated species in all processed flies; finally, the house (45.20%), was the site where the greatest amount of bacterial species was isolated.

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