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ENTEROBACTERIA ISOLATED FROM SYNANTHROPIC FLIES (DIPTERA, CALYPTRATAE) IN MEDELLÍN, COLOMBIA

Enterobacterias aisladas de moscas sinantrópicas (Diptera, Calyptratae) en Medellín, Colombia

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ABSTRACT
Enterobacteria (*Enterobacteriaceae*) causing enteric diseases can be carried and dispersed through insects that act as mechanical vectors, especially flies (Insecta: Diptera). In this study, enterobacteria associated with synanthropic flies were isolated and identified; four different urban areas in the municipality of Medellín were surveyed. Thirteen taxa of calyptrate flies belonging to four families were identified and classified according to the Mechanical Vector Risk Index (MVRI) value, which is proposed in this study. *Chrysomya megacephala* (Fabricius, 1794), *Lucilia cuprina* (Wiedemann, 1819), *Chrysomya albiceps* (Wiedemann, 1819), and *Musca domestica* Linnaeus, 1758 are of high risk; *Fannia* sp., *Atherigona orientalis* Schiner, 1868, and *Ophyra aenescens* (Wiedemann, 1830) of moderate risk; remaining species were classified as low or no risk. *Escherichia coli* was the most frequent bacterium according to the number of isolations (32%), followed by *Klebsiella oxytoca* (12%), *Pasteurella pneumotropica* (11%), and *Kluyvera* spp. (8%). *Raoultella ornithinolitica, Stenotrophomponas maltophilia,* and *Chryseobacterium menigosepticum* were isolated for the first time from flies. Finally, 22 new records of bacteria associated with eight fly species are documented. These results allow us to foresee the existence of a generalist pattern in the interaction between flies and bacteria and indicate that synanthropic flies have a quantifiable potential as vectors of infectious diseases according to the index proposed.

Key words. Bacteria, diarrheal diseases, mechanical vector, risk index.

RESUMEN
Las enterobacterias (*Enterobacteriaceae*) causan enfermedades entéricas. Este tipo de bacterias se pueden transportar y dispersar a través de insectos que actúan como

vectores mecánicos, como las moscas (Insecta: Diptera). En este estudio, se aislaron e identificaron enterobacterias asociadas a moscas sinantrópicas, las cuales se recolectaron en cuatro áreas de diferente uso urbano en el municipio de Medellín. Se identificaron 13 taxones de moscas calipteradas pertenecientes a cuatro familias y se categorizaron según el valor del Índice de Riesgo como Vector Mecánico (IRVM) – propuesto en este estudio –. Las especies *Chrysomya megacephala*, *Lucilia cuprina*, *Chrysomya albiceps* y *Musca domestica* fueron especies de alto riesgo; *Fannia* sp., *Atherigona orientalis,* y *Ophyra aenescens* de riesgo moderado; y las demás se clasificaron como de bajo o ningún riesgo para transportar patógenos entéricos. *Escherichia coli* fue la bacteria más frecuente en los aislamientos (32%), seguida por *Klebsiella oxytoca* (12%)*, Pasteurella pneumotropica* (11%) y *Kluyvera* spp. (8%). *Raoultella ornithinolitica, Stenotrophomponas maltophilia* y *Chryseobacterium menigosepticum,* se aislaron por primera vez asociadas a moscas. Finalmente, se hacen 22 nuevos registros de bacterias asociadas a ocho especies de moscas. Los resultados permiten evidenciar un alto grado de generalismo en la interacción entre moscas y bacterias y que las moscas sinantrópicas tienen un determinado grado potencial como vectores de enfermedades infecciosas de acuerdo con el índice propuesto.

Palabras clave. Bacterias, enfermedades diarreicas, vector mecánico, índice de riesgo.

INTRODUCTION

Enterobacteria are Eubacteria that inhabit the intestinal tract of both humans and animals. They have the ability to cause diseases such as typhoid fever, dysentery and gastroenteritis, which are some of the main causes of mortality and morbidity in the world, where Colombia is not an exception (Manrique *et al*. 2006). They can act as opportunistic pathogens when the host has developed immunosuppression while others exhibit primary pathogenicity and cause infections in healthy people (Patrick 1996). The ability of these bacteria to cause diseases is related to the composition of their outer membrane and sometimes to the presence of a capsule. The membrane contains lipopolysaccharides (LPS), and antigens such as antigen O that confer protection against bacterial cell lysis and phagocytosis and antigen K that acts as adhesins and plays an important role in the virulence (Koneman *et al.* 2004). Furthermore, they have the extracelullar enterotoxins that act as proteases, endoglucanase or hemolysin (Lory 1992). The sources of the infections are mainly related to poor hygiene and sanitary levels, along with the consumption of food contaminated with fecal matter (Manrique & Delfín 1997). It has been proven that flies play an important role in the transmission of enteropathogens to food (Greenberg 1971).

Flies have a great ability to fly and disperse which enables them to be efficient mechanical vectors (Greenberg 1971, 1973). The role of flies in pathogen transmission is mainly related to their biology as decomposers, with polyphagous habits, and endophilic behavior (Sukontason *et al*. 2006); and they carry microorganisms in their digestive tract and the integument (Fetene & Worku 2009). Most flies are not decomposers or scavengers but calyptratae flies in the families: Calliphoridae, Sarcophagidae, Muscidae and Fanniidae are strongly attracted to excrement, secretions and decaying material. They constantly alternate visits between to feces and food, especially species that inhabit human environments (synanthropic flies) (Greenberg 1971, 1973). Polvony (1971) defined Synanthropy as the ability

of some animals to adapt to the conditions made or modified by humans. Flies as synanthropic organisms are classified into the following groups: Eusynanthropic flies that live in human environment, close to or even inside residences; hemisynanthropic flies that live in intermediate environments, between natural and human residences and asynanthropic flies, that live in pristine forests or natural environments (Polvony 1971).

The house fly, *Musca domestica* (Linnaeus 1758) (Diptera: Muscidae) is the most studied species that acts as a mechanical vector and it has been proven to carry and disperse a large number of pathogens, especially gastrointestinal bacteria of the genera *Escherichia, Klebsiella, Enterobacter, Proteus, Enterococcus, Shigella, Salmonella,* and *Aeromonas* (Gupta *et al*. 2012). Although the incidence of flies in the transmission of diarrheal diseases is debatable, it has been reported that control of their populations can be related to the decline of such diseases (Chavasse *et al.* 1999). Diarrheal diseases have a major impact on developing countries such as Colombia where high mortality indices have been recorded (Boschi-Pinto et al 2008).

In some Latin-American countries, such as Venezuela and Brazil, only a few studies on the interaction between bacteria and some particular species of flies have been conducted (Moissant de Román *et al*. 2004) but such studies are lacking in Colombia. The objective of this study was to identify and quantify enterobacteria associated with synanthropic flies (Diptera: Calyptratae) in the city of Medellín, Colombia and provide a basis to comprehend the relationship between bacteria and flies, the potential of these insects to be vectors of enteric pathogens and their connection to the human environment.

MATERIALS AND METHODS

Collection and identification of flies. Fly surveys were conducted in four locations within the urban area of Medellín, Antioquia, Colombia: Marketplace (**MP**) (6°15´26.2 North 75°34´29 West); Residential Complex (**RC**) (6°15´27.6 North 75°34´44.2 West); Municipal Slaughterhouse (**SH)** (6°18´06.4 North 75°33´45.7 West); and an Area of Restaurants (**AR**) (6°12´31.8 North 75°34´04.4 West). Sampling was done twice a month during October, November and December in 2009, and April, 2010. Two modified MacPhail traps (described in Amat 2010) were set at each location, baited with 200g each of decomposing three days old head fish and chicken viscera. This kind of trap allowed us to collect each arriving live fly individually, immediately after it entered the trap. In this way, there was no contact between flies and bait preventing the transfer of pathogens. Each trap was operated from 10: 00 to 14: 00 hours, the peak of activity in several neotropical blowflies species (Baumgartner & Greenberg 1985), with a total sample effort of 4 hours per day, and 8 hours per month.

Each specimen was labeled and stored at room temperature in a sterile plastic bag. Taxonomic identification was performed using the studies of Chillcott (1961), Amat *et al*. (2008), Buck *et al.* (2009), and Buenaventura *et al.* (2009). Additionally, the male genitalia was dissected and macerated in 80% lactic acid ($C_3H_6O_3$) on a hot plate for 20 minutes. Brazilian taxonomic specialists confirmed the species identity of all Muscidae and Sarcophagidae. The mounted specimens were deposited in the entomological collection of Tecnólogico de Antioquia, Medellin, Colombia (CE-TdeA).

Bacterial identification. According to the flies abundance and to make samples size comparable among traps, the first 19 specimens that arrived at the trap at each location were

used to analyze for bacteria. This number was chosen because it was the minimum number of specimens collected in the poorest location during the interval of trap operation. Each selected specimen was deposited in a vial with 5 ml of sterile saline solution (0.9%) to isolate the microorganisms and minimize damage to the external structures of the fly. Ten inversion agitations were performed; then, the specimen was removed for mounting and taxonomic identification and the solution used to inoculate plate-count culture media using calibrated loops of 1 µl and 10 µl. From these cultures, complete colony counting was done in order to quantify the aerobic bacterial load. At the same time, an inoculation of 10 µl in 10 ml of BHI (Brain-Heart Infusion) medium was used to facilitate the retrieval of bacteria for taxonomical purposes. All of the culture media were incubated aerobically at 37 ºC (99 ºF) for 24 hours. After this period, a colony count was performed from the plate-count media. Cultures from the BHI medium were Gram stained and re-streaked on MacConkey media in order to isolate Enterobacteria. After incubation for 24 hours, macroscopic and microscopic characterization (Gram staining) of the isolated colonies were done. Finally, the biochemical identification of the colonies was performed using the API 20 (bioMerieux) Kit, according to the methodology described by the manufacturer.

Data analysis. The bacterial load of each fly specimen was quantified and categorized in three ranges: 0 CFU (Colony forming-units: which is the unit used to estimate the number of viable bacteria cell in the sample); 1-99,999 CFU, >100,000 CFU. The frequency of bacterial species in the isolation procedures was quantified according to the fly species.

Regression and a variance analysis of the bacterial load were performed using a general linear model using SPSS software (Pardo & Ruiz 2002) in which the dependent variable was CFU and independent variables were the genus, species, and location; the effects were analyzed using the F test. An analysis of main coordinates was performed based on bacterial presence-absence data in each fly species. To compare pairs of fly species, a Sokal & Michener's (1958) simple matching (SM) coefficient was calculated for each comparison. This coefficient considers the occurrence and absence of bacterial species depending on fly host. A fly species similarity matrix was calculated and the graphs were generated using Matlab® 7 software (Moler 2004).

To quantify the potential risk of flies as bacterial carriers, a Mechanical Vector Risk Index (MVRI) is proposed. Unlike the first index proposed by Mihályi (1967) and then modified by Maldonado & Centeno (2003) based on behavioral patterns, synantropy degree and relative body size among others; most of them unknown for many neotropical species. The MVRI here proposed considers only three key aspects; the ubiquity, bacterial load and bacterial richness associated with each fly species found using data taken directly from field work. MVRI will allow us to categorize each fly, determine risk, identify potential dangerous species and made coherent decisions to establish control and sanitary measures.

The index is defined by the following equation:

$$\mathbf{MVRI} = \left[\left(\frac{a}{A}\right) \mathrm{x} \left(\frac{b}{B}\right) \mathrm{x} \left(\frac{c}{C}\right)\right]$$

where a = number of locations where the fly species was found, A = total number of sampled locations, b = number of isolation events of bacteria with a CFU > 100 000 at 1µl, B = total number of isolation events of bacteria at 1µl, c = number of species of bacteria isolated in the fly specie, C= total number of species of bacteria isolated in the study.

The first term of the equation explains the degree of ubiquity and spatial occurrence of the fly taxa assessed. The value range is between 0 and 1, with 1 being a fly species widespread in all locations of collection. The second term of the equation treats the levels of bacterial load in terms of quantity of CFU at the lower level of dilution (1 µl), a 1 of one being a fly species where all cases of isolation were higher than 100 000 CFU. The last term is the bacterial richness. This value ranges from 0 to 1, with 1 being a fly that carried all bacterial species richness found in the study. We proposed four risk categories based on MVRI: none, low, moderate and high; value ranges by proposed category were calibrated according the preexisting information of the species *Musca domestica* which has been widely studied and considered as an efficient pathogen carrier with high risk (Sukontason et al. 2006). *M. domestica* MVRI value was 0.09, we adopted this value to establish the lower limit of the high risk category (up to 1). Then if the observed index = 0, the fly has no risk as mechanical vector; species with values ≥0.01 and <0.03 were considered low risk species; species with values ranging ≥0.03 to <0.09 were moderate risk species and with values ≥0.09 to 1 were high risk species as pathogen carriers. Specific values may change due to the biogeographic area assessed; this could be more realistic considering the different behavior, bionomics and synantropic degree of individuals belonging to the same species in different geographical localities (Greenberg 1971). In future studies the calibration of each category here proposed must be performed again following the values of any high risk reference fly species (in this case *Musca domestica*) commonly found.

RESULTS

Collection and identification of flies. Thirteen fly taxa were identified (Table 1); nine at the species level, eight recorded as synanthropic (Greenberg 1973) and one, *Hemilucilia semidiaphana* (Rondani 1850) (Diptera: Calliphoridae) reported as asynanthropic (Baumgartner & Greenberg 1985). All of them have been recorded previously in Medellín (Salazar-Ortega *et al*. 2012). The fly species ubiquity is showen in table 2.

Table 1. Enterobacteria species isolated from fifteen synanthropic species of flies from 4 locations and frequency of bacteria occurrence per isolation case, from October 2009 to April 2010 in Medellin, Colombia.

Taxon	Location	Enterobacteria isolated	Frequency (# cases of each specie / total bacterial species)
Calliphoridae			
Lucilia eximia (Walker, 1849)	RC	*Escherichia coli* (Migula, 1895)	*1/1*
Lucilia cuprina (Wiedemann, 1819)	RC, AR, MP, SH	*Klebsiella oxytoca* (Flügge, 1886)* *Kluyvera sp.** *Escherichia coli* (Migula, 1895) *Raoultella ornithinolytica*†* (Sakazaki *et al.*, 1989) *Pasteurella multocida 1*†* (Lehmann & Neumann, 1899) *Enterobacter cloacae** (Jordan, 1890) Hormaeche & Edwards, 1960	*2/10* *1/10* *4/10* *1/10* *1/10* *1/10*
Lucilia sp.	RC, AR, SH	*Klebsiella oxytoca* (Flügge, 1886) Lautrop, 1956	*1/1*
Chrysomya megacephala (Fabricius, 1794)	RC, AR, MP, SH	*Escherichia coli* (Migula, 1895) *Providencia rettgeri* (Hadley *et al*., 1918) Brenner *et al*., 1978 *Pasteurella pneumotropica* (Jawetz, 1950) *Kluyvera* sp.* *Serratia odorifera 1** (Grimont *et al*., 1978) *Chryseobacterium meningosepticum*†* (King, 1959) Vandamme *et al*., 1994 *Enterobacter sakazakii** (Farmer *et al*., 1980)	*1/7* *1/7* *1/7* *1/7* *1/7* *1/7* *1/7*

Continued Table 1. Enterobacteria species isolated from fifteen synanthropic species of flies from 4 locations and frequency of bacteria occurrence per isolation case, from October 2009 to April 2010 in Medellin, Colombia.

Taxon	Location	Enterobacteria isolated	Frequency (# cases of each specie / total bacterial species)
Chrysomya albiceps (Wiedemann, 1819)	RC, AR, SH	*Escherichia coli* (Migula, 1895) *Klebisella oxytoca** (Flügge, 1886) *Pasteurella pneumotropica** (Jawetz, 1950) *Kluyvera* sp.* *Providencia rettgeri** (Hadley *et al*., 1918) Brenner *et al*., 1978 *Providencia stuartii** (Buttiaux *et al*.1954) Ewing, 1962	*3/9* *1/9* *1/9* *1/9* *1/9* *1/9*
Cochliomyia macellaria (Fabricius, 1775)	SH	*Escherichia coli* (Migula, 1895)	*2/2*
Hemilucilia semidiaphana (Rondani, 1850)	AR	*Klebsiella oxytoca** (Flügge, 1886) *Pasteurella pneumotropica** (Jawetz, 1950)	*1/2* *1/2*
Muscidae			
Ophyra aenescens (Wiedemann, 1830)	MP, SH	*Pasteurella pneumotropica** (Jawetz, 1950) *Serratia odorifera 1**(Grimont *et al*., 1978)	*1 /2* *1/2*
Musca domestica (Linnaeus, 1758)	RC, AR, MP, SH	*Escherichia coli* (Migula, 1895) *Klebsiella pneumoniae* (Schroeter 1886) Trevisan, 1887 *Pasteurella multocida 2*†* (Lehmann & Neumann, 1899) Rosenbusch & Merchant, 1939	*1/3* *1/3* *1/3*
Atherigona orientalis (Schiner, 1868)	RC, MP, SH	*Escherichia coli** (Migula, 1895) *Pasterurella pneumotropica** (Jawetz, 1950)	*1 /2* *1 /2*
Sarcophagidae			
Peckia sp.	RC, MP	*Escherichia coli* (Migula, 1895)	*1/1*
Oxysarxodexia sp.	RC, MP	*Klebsiella oxytoca* (Flügge, 1886)*	*1/1*
Fanniidae			
Fannia sp.	RC, AR, MP, SH	*Escherichia coli* (Migula, 1895) *Klebsiella pneumoniae* (Schroeter, 1886) Trevisan, 1887 *Raoultella ornithinolytica*†*;*(Sakazaki *et al*., 1989) *Stenotrophomonas malthopila*† (Hugh, 1981) *Aeromonas hydrophila* (Chester, 1901) Stanier, 1943 *Enterobacter cloacae* (Jordan, 1890) Hormaeche & Edwards, 1960	*1/7* *2/7* *1/7* *1/7* *1/7* *1/7*

(†) Isolated for the first time from a fly, (*) New record of bacteria associated with the fly species. AR: Area of Restaurants; MP: Marketplace; RC: Residential Complex; SH: Municipal Slaughterhouse.

Bacterial identification. Sixteen bacteria species were isolated from theses flies (Table 1); *E. coli* had the highest frequency of all isolations (32%) (Fig. 1). The highest frequency number of positive isolation at 1 µl and 10 µl was observed for >100 000 CFU, followed by 0 CFU (Fig. 2).

Regression and variance analyses. According to the regression and variance analyses of the CFU at 1 µl dilution, differences were significant only for fly genus and location ($p \leq 0,05$). Thus most of the genera of flies (except *Chrysomya*) collected in the marketplace had a bacterial load > 100.000 CFU, while in the residential complex, several genera of flies (*Atherigona* sp., *Chrysomya* sp., *Fannia* sp., *Lucilia* sp., *Musca* sp., *Oxysarcodexia* sp., *Peckia* sp.) had no bacterial growth.

Analysis of main coordinates. The analysis of main coordinates (AMC) (Fig. 3) is interpreted depending on the location of fly on the graph. Species of the sample observed with normal values are located in the center, and those that are away from this had higher bacterial load, more types of bacteria, and therefore a greater risk index as mechanical vectors. This procedure shows a relationship between *Chrysomya albiceps* (Wiedemann 1819) and *Chrysomya megacephala*. The specimens of *Cochliomyia macellaria* (Fabricius 1775) (Diptera: Calliphoridae), *Peckia* sp., *Ophyra aenescens* (Wiedemann 1830) (Diptera: Muscidae), *Lucilia eximia* (Walker 1849) (Diptera: Calliphoridae), *Lucilia* sp., *Atherigona orientalis* (Schiner 1868) (Diptera: Muscidae), and *H. semidiaphana* form a group of close proximity. Affinities

between the species *M. domestica* and *Lucilia cuprina* (Wiedemann 1819) (Diptera: Calliphoridae), which are cosmopolitan and synanthropic, are also evidenced. *Fannia* sp. is separated from the rest of the group (Fig. 3).

Risk index values of flies. According to the risk index values (Table 2), *L. cuprina, C. megacephala*, *C. albiceps*, and *M. domestica* had values ≥ 0.09, which led us to consider these as high risk species. On the other hand,

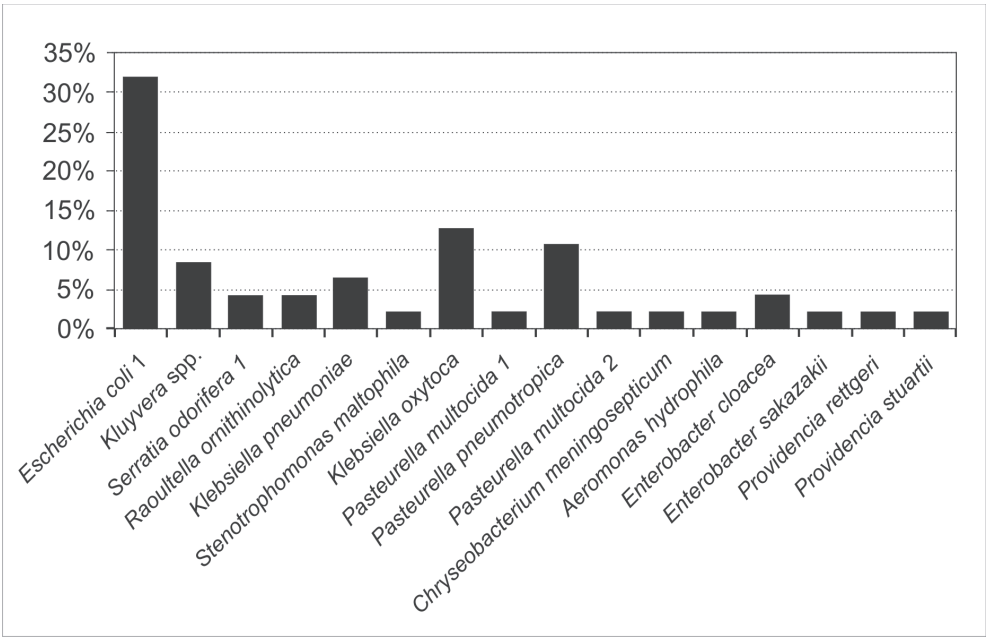

Figure 1. Values of relative frequency of Enterobacteria occurrence by isolation cases from synanthropic flies in Medellín, Colombia.

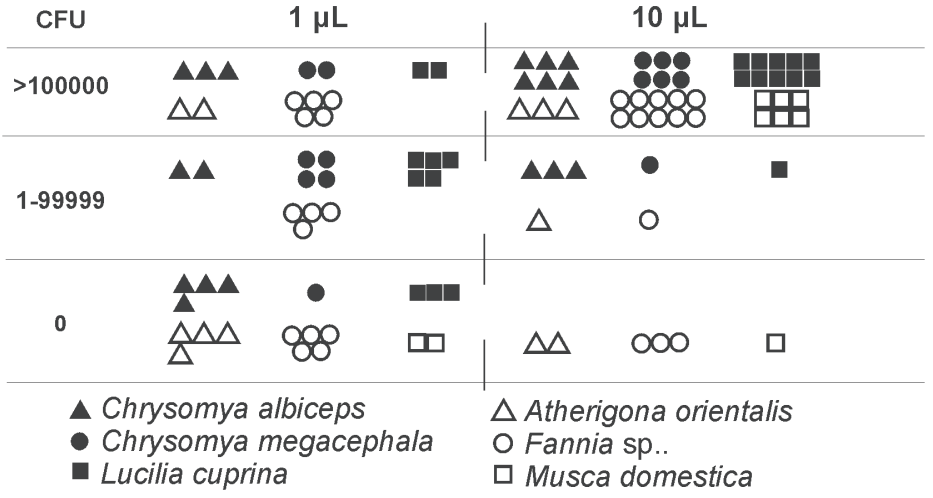

Figure 2. Frequency of bacteria isolation cases per amount of CFU (Unit Forming Colony) at 1 and 10 ul by fly species.

A. orientalis, *O. aenescens* and *Fannia* sp. showed values between 0.03-0.07, considered here as a moderate risk. The remaining taxa with index values < 0.03 units, are considered here to be low or no risk taxa. The grouping and spatial arrangement in the main coordinates graph (Fig. 3) also reflects the MVRI trend values of each species.

DISCUSSION

Our study shows that fly body surfaces can carry several bacterial species but the bacteria are not specific to species of fly, nor did we find evidence of any specificity between the categories of bacterial load and the type of fly (species or genera); individual fly specimens have differences in terms of bacterial load depending on the genera they belong to and the locality where they were collected, and bacterial load can vary within individuals of the same species (Fig. 3; Table 2). This suggests that the contact of the fly with food might be enough to produce contamination that causes an infection (Manrique & Delfín 1997), and may be associated with bacteremia, septicemia, intestinal infections and nosocomial diseases (Bellier *et al.* 2008).

Bacterial identification. Many of the bacteria found in this study are known to cause many diseases (Table 3). Of these, *Pasteurella multocida*, is part of the oropharyngeal and gastrointestinal flora

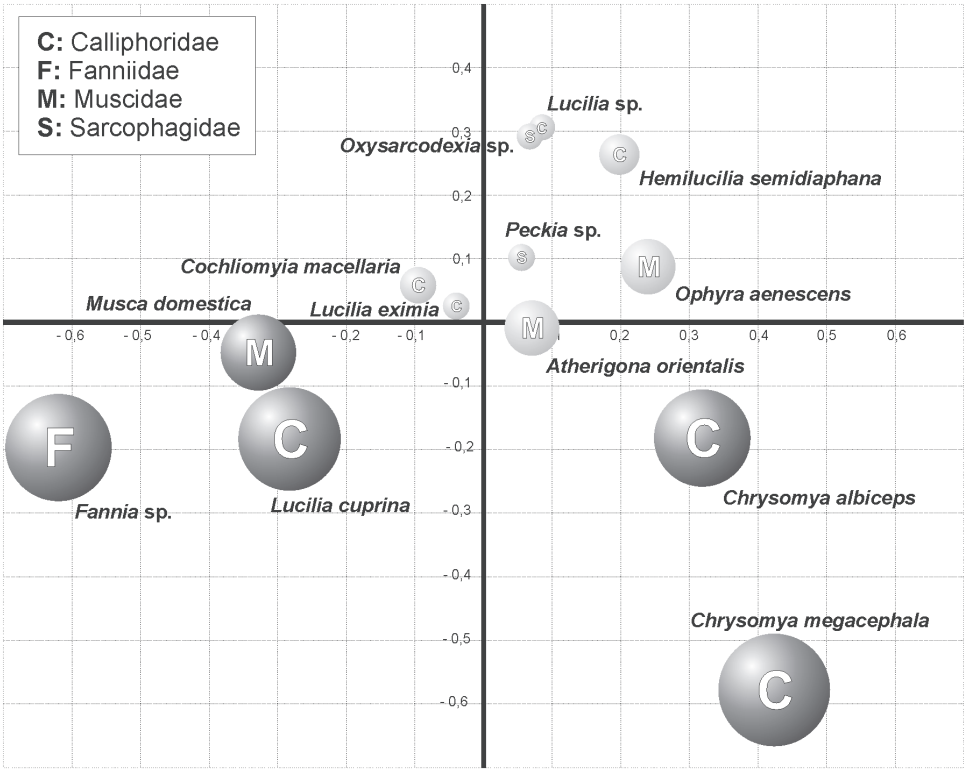

Figure 3. Grouping and spatial arrangement of the fly species according to their bacterial richness in the analysis of main coordinates. Each sphere represents a fly species. Its location on the graph represents the similarity in terms of bacterial loads and kind of bacterium carried. The sphere size is proportional to the mechanical vector risk index value.

of dogs and cats (Weber *et al*. 1984). The isolation of this bacterium from flies allows us to infer a direct relationship between flies and the feces of rodents, cats and/or dogs. The bacterium *Raoultella ornithinolitica* has been found before in the alimentary tract of fruit fly species of the genus *Bactrocera* spp. (Thaochan *et al*. 2010). *Stenotrophomonas maltophilia* was previously recorded in the digestive tract of the sand fly *Lutzomyia longipalpis* (Lutz & Neiva) by Gouveia *et al*. (2008). *Chryseobacterium menigosepticum* is here recorded for the first time as associated with the surface of flies.

From the bacteria isolated, *Citrobacter*, *Enterobacter*, *Escherichi*a, *Klebsiella*, *Aeromonas*, and *Pasteurella* are taxa that have been recorded previously in *Musca domestica* (Moissant de Román *et al*. 2004). Also *Aeromonas* was isolated from *Chrysomya megacephala* (Fabricius 1794) the oriental latrine fly (Diptera: Calliphoridae) (Bunchu 2012). *Serratia, Klebsiella, Enterobacter, Kluyvera, Providencia,* and *Stenotrophomponas* have been isolated from nematocerous dipterans (Luz-Alves *et al*. 2010). The genera *Serratia* and *Enterobacter* have also been reported in ants (Hymenoptera: Formicidae) and cockroaches (Blattodea) collected in hospitals and residences (Prado *et al*. 2002).

Regression and variance analyses. According to regression and variance analyses results, we can predict that flies of the same genus probably shared similar foraging behavior; therefore the bacterial load could be similar. In this case the bacterial load of specimens greatly depends on the location where the fly was collected.

Analysis of main coordinates. The analysis of main coordinates (AMC) (Fig. 3) shows a relationship between fly species suggesting that these have an affinity for the same substrate. This is in agreement with the regression and variance results, and habits reported in previous studies. (Wijesundra 1957, Martínez - Sánchez *et al*. 2000, Linhares 1981). Adults of *C. megacephala* have been

Table 2. Values and quantified parameters for the evaluation of the potential risk as mechanical vectors in synanthropic flies from Medellín, Colombia.

Species	Ubiquity		Bacterial load		Bacterial richness	Index Value	Level of risk
	Number of locations (n=4)	(%)	Number of isolations with a CFU >100,000 (1ul)/ Total number of isolations	(%)	Spp. number of isolated bacteria	(MVRI)	
Atherigona orientalis (Schiner, 1868)	3	75	2/6	33.3	2	0,03	Moderate
Chrysomya albiceps (Wiedemann, 1819)	3	75	3/9	33.3	6	0,09	High
Chrysomya megacephala (Fabricius, 1794)	4	100	2/7	28.5	7	0,13	High
Cochliomyia macellaria (Fabricius, 1775)	1	25	1/2	50	1	0,01	Low
Fannia sp.	4	100	5/14	35.7	6	0,075	Moderate
Hemilucilia semidiaphana (Rondani, 1850)	1	25	0/2	0	2	0	None
Lucilia cuprina (Wiedemann, 1819)	4	100	3/11	27.3	6	0,1	High
Musca domestica (Linnaeus, 1758)	4	100	3/7	42	3	0,09	High
Ophyra aenescens (Wiedemann, 1830)	2	50	1/2	50	2	0,03	Moderate
Lucilia eximia (Wiedemann, 1819)	1	25	1/1	100	1	0.02	Low

found in slaughterhouses, and around meat, fish, candy and other foodstuffs. Furthermore, they have been observed near decaying animal carcasses and breeding in garbage (Wijesundra 1957) and excrement. Similarly, adults of *C. albiceps* feed on feces, carrion and rotting fruit (Martínez-Sánchez *et al*. 2000). *C. megacephala* and *C. albiceps* showed a high frequency per sampling site, a high bacterial load, and the highest number of species of associated bacteria. These parameters already included in the MVRI (Fig. 3; Tables 1-2) suggest that exotic *Chrysomya* spp. have the ability and the potential to be more effective vector of enteric disease than do the native species.

Affinities between the species *M. domestica* and *Lucilia cuprina*, are also revealed. These species are common in homes and places where food is handled (Linhares 1981). This clustering may be related to feeding and breeding behaviors, which is confirmed by the frequency with which these species are observed flying in restaurants and homes (endophilic habits) in Medellin (this species was previously collected in restaurants and identified by E. Amat). On the other hand, *Fannia* sp. is separated from the rests of the flies groups. Species belonging to this genus have been found in a great variety of substrates and environments. Some of them breed in chicken excrement, others have been recorded around marketplaces, under remains of grass-covered wood, in crevices of tree trunks, holes in walls of buildings, toilets and near plants and fruits, to which they are attracted by honey dews and sap (Savage 2010). Although there are records of *Fannia* invading homes, Chillcott (1961) observed that the species of this genus do not easily colonize human environments. The larvae of these flies are found in animals and decaying vegetable matter, especially in feces, and sometimes, in bird nests.

Risk index categories of flies. According to the risk index values, *L. cuprina, C. megacephala*, *C. albiceps*, and *M. domestica* have the highest values. These findings are in agreement with Maldonado & Centeno (2003) in Argentina. Although several criteria were included in the index, we have no certainty that a high value corresponds with a high rate of enteric infection causing pathologies. It is highly recommended to study the frequency of clinical cases caused by enterobacteria and the occurrence, frequency and monitoring of carriers flies; also to investigate seasonal patterns in order to identify variations in the assemblages of the fly communities. It is possible to discriminate and classify flies according to conditions involving the mechanical transmission indicated by Greenberg (1971): the degree of synantropy depends on the flight activity and dispersal in

Table 3. List of diseases caused by bacteria species isolated in this study.

Specie of bacteria	Diseases	Reference
Escherichia coli	Extraintestinal, intestinal and diarrheal infections	Hannaoui *et al*. 2010, Saha *et al*. 2013
Citrobacter freundii	Meningitis	Plakkal *et al*. 2013
Kluyvera spp	Meningitis	Paredes *et al.* 2002
Enterobacter sakazakii	Meningitis	Stoll *et al*. 2004
Klebsiella pneumoniae	Pneumonia	Wang *et al*. 2010
Aeromonas hidrophyla	Pneumonia, extraintestinal infections	Mukhopadhyay *et al*. 2003, 2008
Enterobacter cloacae	Otitis	Pino *et al*. 2003
Providencia rettgeri	Ocular infections, urinary tract infections	Koreishi *et al*. 2006
Pasteurella multocida	Skin and soft tissue infections, respiratory tract infections	Weber *et al*. 1984, Sánchez *et al.* 2009
Chryseobacterium menigosepticum	Meningitis, nosocomial infection, bacteremia, abdominal infection and also necrotizing fasciitis	Hsu *et al.* 2011

terms of ubiquity; the consumption of both contaminated and non-contaminated food and the alternation with carrion, feces and organic matter in decomposition is expressed here and correspond with the amount of bacterial load and species richness.

The species *C. megacephala*, *L. cuprina, C. albiceps*, and *M. domestica* had the highest synanthropy index value in earlier studies (Baumgartner & Greenberg, 1985). This association represents a major risk to public health, as they are carrying agents capable of transmitting enteropathogenic bacteria to food. On the other hand, *H. semidiaphana* has previously been reported as asynanthropic (SI= -73) in Brazil and Peru (Baumgartner & Greenberg 1985) but in this study it demonstrated an obvious and previously unknown synanthropic tendency. However this species had the lowest risk index in this study, due to a null isolation event of bacteria with CFU >100,000 and consequently the MVRI value equal to zero, this means that at least for Medellin, *H. semidiaphana* despite being a synanthropic fly has no importance as an enterophatogen carrying vector. However its medical importance may need to be assessed carefully if it behaved as eusynanthropic fly in other localities as it is attracted to human feces in Peru (Baumgartner & Greenberg 1985).

C. macellaria is widely known in the tropics as an efficient mechanical vector of pathogens, with endophilic, hemisynantrophic or eusynantropic behaviour and is strongly attracted to human feces and feeds on carrion, fish, fruit and garbage (Baumgartner & Greenberg, 1985). In this study this species was found only in one location, without considerable bacterial load, and according the MVI value and risk category here assigned *C. macellaria* seems to have little medical importance in contrast to the above mentioned studies. We recommend that this species is assessed at others localities and habitats as it may have different bionomic responses in each case.

The results allow us to foresee the existence of a high degree of generalism in the interaction between flies and bacteria. The amount and type of bacteria associated with a specific fly greatly depend on the genus of fly and the geographic location. The impact of the bacteria mentioned above on public health, and the risk posed by the association of these bacteria with synanthropic flies indicate that these insects have great potential as vectors of infectious diseases. Studies to evaluate the potential of these flies as mechanical vectors by means of the relationship among their degree of infection, the presence of enterobacteria and the pathology of patients with diarrheal episodes are recommended. Studies in relation to viruses, protozoos and helminthes on flies should be also considered.

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