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Isolated bacteria from hematophagous Culicidae (Diptera: Nematocera) in Belém, Pará State, Brazil*

Bactérias isoladas de culicídeos (Diptera: Nematocera) hematófagos em Belém, Pará, Brasil

Bacterias aisladas de culícidos (Diptera: Nematocera) hematófagos en Belém, Pará, Brasil

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ABSTRACT

Bacteria are largely distributed in nature, especially when carried by a vector. They comprise large portions of the human and animal microbiota, and some cause diseases. The diptera of the family Culicidae are directly involved in the vectoring of epidemics of great interest for public health. However, the association between bacteria and Culicidae has been scarcely studied. In order to deepen the knowledge on this subject, we isolated and identified bacteria which have been transported in hematophagous Culicidae in the City of Belém, Pará State. The collection of 296 mosquitoes was carried out using a CDC light trap in eight collection localities, which presented different environmental characteristics within the metropolitan area of Belém. Some were identified to the species level (9) and others to the subgenus (4). It was possible to identify 17 species of bacteria; seven bacteria could only be identified up to their genus. *Culex quinquefasciatus* and *Anopheles aquasalis* were the most frequent Culicidae. The most frequent species of bacteria found in the samples were *Gemella haemolysans* and *Enterobacter cloacae*. The collection localities in the Terra-Firme and Curió-Utinga districts presented the largest diversity of species of Culicidae.

Keywords: Bacteria; Culicidae; Biological Transport; Amazonian Ecosystem.

INTRODUCTION

The order Diptera, which includes flies and mosquitoes, has close to 150 thousand species and is the fourth largest of the class Insecta. Its members occupy various niches in different aerial, aquatic, and terrestrial habitats. The order Diptera includes different families of medical interest in the suborder Nematocera (Culicidae, Ceratopogonidae, Simuliidae and Psychodidae). In Brazil, there are approximately 20,000 dipterous species in approximately 100 families listed in the Catalogue of the Neotropical Region[42], which is still not complete. The Diptera in Brazil are still not well understood and are proposed to encompass about two to three times more species than currently recorded[8].

There are about 3,600 species in the family Culicidae that have a worldwide distribution. These compose approximately 40 genera, with the neotropical species having the highest level of endemism, as 27% of these groups are restricted to this biogeographical region. Little is known about the Culicidae fauna of the Amazon. The last study on the distribution of mosquitoes that covered the entire Amazon region was carried out in 1961[9] and collected 218 species, 152 of which were in the State of Pará[51]. Mosquitoes comprise a vast group of insects, containing many genera and species. From the perspective of human health, the most important genera are *Anopheles, Aedes* and *Culex*.

There are few studies published worldwide on the relationship between bacteria and dipterous with even fewer on bacteria and Culicidae. Only in the last ten years has research on this relationship begun to emerge and gain importance in the scientific community. Bacteria have been identified in the digestive tract of various insect species that constitute the intestinal microbiota[40]. Studies performed with mosquitoes bred in insectariums in Mexico[20] and Brazil[24] showed the presence of Gram-negative bacteria in their intestines. Some bacteria are being used as biological pest control of adult insects and larvae in plantations[13,49,5,50], while others are being tested as an alternative to controlling

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mosquito populations that cause epidemics[43,30,14,3]. Some other studies are evaluating the association between bacteria with mechanical vectors and the possibility of transmission by various insects[31,41,11,37,44]. In a 2007 study[31], more than 20 bacteria species were isolated from horseflies and preserved, including those belonging to the *Staphylococcus, Streptococcus* and *Serratia* genera.

The process of city planning contributes to the distribution of dipterous species. Environments that are more heavily populated contain species with a greater ability to adapt to these sites, whereas areas with greater forestation and less human influence naturally select other dipterous species. The flies, therefore, are separated in niches within the same city. The bacteria that the flies carry are also varied and previous research does not elucidate the role of the bacteria in this problem. There are few studies on this subject in Brazil and further studies for the advancement of knowledge in this area are necessary. The main objective of this paper is to evaluate the entomological and bacteriological diversity associated with distinct urban areas in the city of Belém.

METHODOLOGY

AREAS OF STUDY

In the City of Belém, seven collection points were selected with different urbanizations characteristics. The eighth point was selected in an area near the banks of the Pará River estuary, located in Outeiro, an administrative district in Belém.

Central area of Belém

The inner city area of Belém, characterized by the existence of many houses and buildings and few areas of bare soil. It comprises many cemented and paved areas, and vegetation is restricted to gardens and ornamental flora of the city. The selected sampling points were located in the Cremação and Nazaré neighborhoods.

Pericenter of Belém

This area is characterized by many houses and few buildings, with areas of bare ground and little cement and asphalt. Most vegetation is in backyards and less ornamental vegetation exists here than in the downtown. The selected sampling points were located in the Curió-Utinga and Jurunas neighborhoods.

Outskirts of Belém

This is an area with few houses and no buildings where houses are separated by empty lots. There are soil lots without cement and asphalt, and abundant vegetation on uninhabited land and in backyards. Stretches of forests can still be found in this area. The selected sampling points were located in the neighborhoods of Tapanã, Terra Firme and Icoaraci.

Estuary Area

The floodplain of the Para River estuary is a distinct ecosystem with unique biotic and abiotic characteristics. We propose that hematophagous insects and their relations to bacteria influence the dynamic properties of this ecosystem. Collections were made in the estuary of the Pará River in Outeiro.

COLLECTION OF CULICIDAE

Insects were collected from May 2007 to April 2008, from 17 h until 22 h or until 6 h the next day. Samples were collected with appropriate techniques to minimize contamination of the traps and containers used for collecting Culicidae.

We used CDC light traps[47], which are generally used for sampling of hematophagous insects (especially Culicidae, phlebotominae and ceratopogonidae). These traps attract insects to a small tungsten light source. When these small insects are close enough to the light, they are sucked into the trap by a small fan that is driven by a 12 V current. Using this method, the collected insects remain alive until they are removed from the apparatus.

IDENTIFICATION OF THE CULICIDAE

After being collected, the mosquitoes were immediately taken to the Department of Arbovirology of the Instituto Evandro Chagas (IEC), Belém, Pará. With the help of specialists, we identified the specimens using identification keys cited by three classic articles on taxonomy of Culicidae: Foranttini[19], Gorham et al[21] and Faran and Finthicum[17].

IDENTIFICATION OF BACTERIA

After the mosquitoes were identified, they were immediately treated for the detection of bacteria.

The mosquitoes were separated into groups (pools) according to the number of specimens that were collected from each species. We studied a total of 41 pools with three specimens and six pools with two specimens for a total of 129 Culicidae. In some cases, pools with two specimens were used because of insufficient samples collected from a particular species.

To create the pools, the mosquitoes were separated aseptically (near a Bunsen burner) using a biological safety cabinet. There was no direct handling of the collected Culicidae specimen in any stage of the research; specimens were placed in individual test tubes, which facilitated identification and reduced sources of contamination.

After the groups were identified and defined, a suspension of the mosquitoes was prepared by grinding them in a mortar with a sterile physiological solution. Next, an aliquot was withdrawn and inoculated in one of two culture media, the Tryptic Soy Broth (TSB) and sodium thioglycollate, at 37° C for 24 h.

An aliquot of the material contained in the tubes of TSB and thioglycollate where growth (turbidity) had been observed was thereafter plated onto blood agar in 5 to 10% CO_2, Chapman agar, and MacConkey agar. After plating, these were then incubated at 37° C for 24 h.

The colonies grown on blood agar and Chapman agar were submitted to a bacterioscopy by the Gram stain method and the Gram-positive cocci and bacilli were identified[28]. Three to five colonies from MacConkey agar were plated onto a sorting TSI (triple sugar and iron) medium and the Gram-negative bacilli were thereafter identified[15,25].

For the biochemical characterization, the ID 32 E, API 20 E, API 50 CH, API Staph, API Corine and API 20 Strep systems were also applied using the API Bio Mini apparatus (Mérieux, France). The quality control of the kits for biochemical determinations was performed using the standardized samples ATCC-25922 *E. coli*, ATCC-27853 *P. aeruginosa* and ATCC-25923 *S. Aureus*.

RESULTS

We collected a total of 296 hematophagous Culicidae throughout the study period, but not all specimens were used for the bacteriological study. Only 129 were divided into 41 pools of three specimens of the same species and three pools containing two specimens. Most of the Culicidae collected were identified at the species level, including *Culex* (*Culex*) *coronator*, *Anopheles* (*Nyssorhynchus*) *triannulatus*, *Coquillettidia* (*Rhynchotaenia*) *venezuelensis*, *Mansonia* (*Mansonia*) *titillans*, *Culex* (*Culex*) *quinquefasciatus*, *Mansonia* (*Mansonia*) *titillans*, *Aedes* (*Stegomyia*) *aegypti*, *Anopheles* (*Nyssorhynchus*) *aquasalis* and *Psorophora* (*Janthinosoma*) *ferox*. Other specimens, however, were identified only at the genus level, including *Culex* (*Culex*) spp., *Phoniomyia* spp. and *Culex* (*Melanoconion*) spp.

Out of all the mosquitoes collected, only *Psorophora* (*Janthinosoma*) *ferox* and *Phoniomyia* spp. showed no bacteria growth in the medium selected for identification. However, bacterial growth only appeared in specimens collected from Nazaré when comparing *Anopheles* (*Nyssorhynchus*) *aquasalis* specimen collected at the Nazaré and Outeiro points.

The *C. quinquefasciatus* species was the most frequently collected (85 samples), representing 28.7% of the total mosquitoes collected (Figure 1). We collected 42 specimens of *A. aquasalis* and 40 specimens of *A. aegypti*, which represented 14.1% and 13.5% of the mosquitoes, respectively. The Terra Firme sampling site had the highest number of Culicidae species, where it was possible to identify the following specimens at the species level: *Coquillettidia venezuelensis*, *Ochlerotatus serratus* and *Psorophora ferox*. The subgenera *Culex* (*Culex*) spp., *Trichoprosopon* (*Trichoprosopon*) spp., and the *Phoniomyia* spp genus were identified. The Curió-Utinga collection point also contained a large number of Culicidae; at this site, four specimens were identified at the species level and one at the subgenus level. In the Cremação neighborhood, only the *Culex quinquefasciatus* species was collected.

Out of the collected Culicidae, 17 species and seven genera of bacteria were identified. Among the identified bacteria, the following species were dominant: *Gemella haemolysans*, *Enterobacter cloacae* and *Enterococcus faecalis* (Figure 2), which represented 14.5%, 12.3% and 8.9% of the total identified bacteria, respectively. The *Staphylococcus* genus (negative in the coagulase test) was identified in 10% of the samples analyzed. Figure 3 illustrates the number of bacteria species in each of the mosquito collection points. *Culex quinquefasciatus*, *Coquillettidia venezuelensis* and *A. aegypti* were the Culicidae species that contained the greatest number of bacteria species. There was no bacterial growth in culture media with specimens of *Psorophora ferox* and *Phoniomyia* spp.

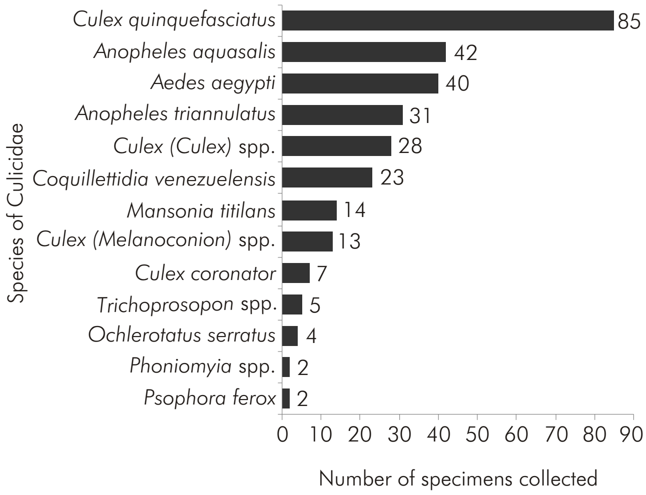

Figure 1 – Culicidae species with respective numbers of collected specimens

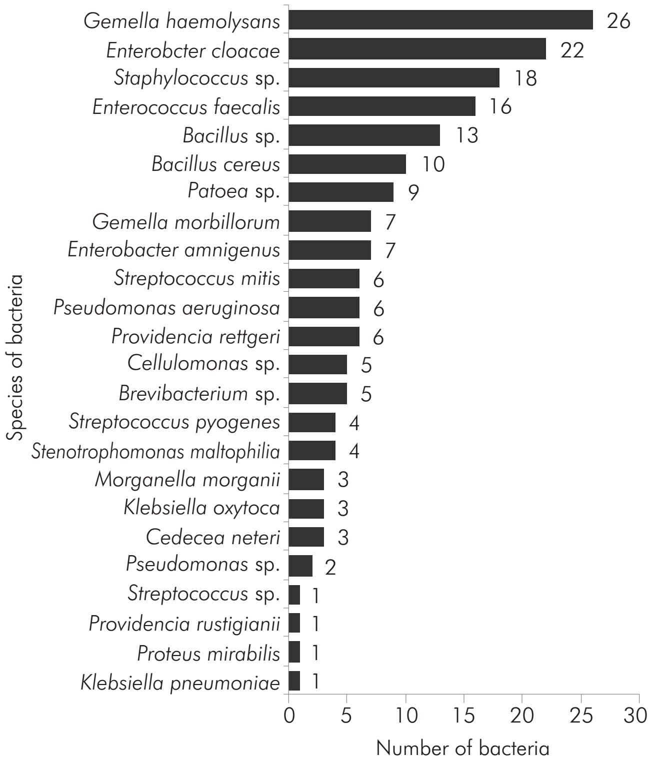

Figure 2 – Number of occurrences of bacteria species in samples

Table 1 presents the obtained results relating the isolated bacteria to the Culicidae species and showing the frequency of bacteria in each Culicidae species to the points where they were collected. It was observed that *E. cloacae* was found in at least 6 species of Culicidae while *G. haemolysans* and *Staphylococcus* sp. were present in at least four species. *B. cereus* and *Phatoes* sp. were found in

Table 1 – Frequency of bacteria identified in mosquitoes collected in the Belém collection points

Species of bacteria	Species of Culicidae	Collection locality	f (%) of bacteria
Bacillus cereus	*Culex (Culex)* spp.	Curió-Utinga	5.59
	Culex (Culex) coronator Dyar and Knab		
	Anopheles (Nyssorhynchus) triannulatus s.l. (Neiva and Pinto)		
Bacillus sp.	*Anopheles (Nyssorhynchus) triannulatus* s.l. (Neiva and Pinto)	Nazaré	2.79
	Coquillettidia (Rhynchotaenia) venezuelensis (Theobald)	Curió-Utinga	4.47
Cedecea neteri	*Mansonia (Mansonia) titillans* (Walker)	Curió-Utinga	1.25
	Culex (Culex) quinquefasciatus Say	Cremação	0.56
Cellulomonas sp.	*Culex (Culex)* spp.	Curió-Utinga	2.79
	Culex (Culex) coronator Dyar and Knab		
Enterobacter amnigenus	*Culex (Culex) quinquefasciatus* Say	Cremação	0.56
		Icoaraci	1.67
	Culex (Melanoconion) spp.	Nazaré	1.67
Enterobacter cloacae	*Mansonia (Mansonia) titillans* (Walker)	Curió-Utinga	2.23
	Culex (Culex) coronator Dyar and Knab		
	Coquillettidia (Rhynchotaenia) venezuelensis (Theobald)		
		Terra-Firme	1.12
	Culex (Culex) quinquefasciatus Say	Cremação	3.91
	Culex (Melanoconion) spp.	Nazaré	3.35
	Aedes (Stegomyia) aegypti (Linnaeus)	Icoaraci	1.67
Enterococcus faecalis	*Culex (Culex) quinquefasciatus* Say	Cremação	6.14
		Jurunas	2.79
	Aedes (Stegomyia) aegypti (Linnaeus)		
Gemella haemolysans	*Ochlerotatus (Ochlerotatus) serratus (Theobald)*	Terra-Firme	7.82
	Trichoprosopon (Trichoprosopon) spp.		
	Anopheles (Nyssorhynchus) aquasalis Curry	Nazaré	5.02
	Aedes (Stegomyia) aegypti (Linnaeus)	Jurunas	1.67
Gemella morbillorum	*Culex (Culex)* spp.	Terra-Firme	3.91
	Coquillettidia (Rhynchotaenia) venezuelensis (Theobald)		
Klebsiella oxytoca	*Coquillettidia (Rhynchotaenia) venezuelensis* (Theobald)	Curió-Utinga	1.67
Klebsiella pneumoniae	*Aedes (Stegomyia) aegypti* (Linnaeus)	Tapanã	0.56
Morganella morganii	*Ochlerotatus (Ochlerotatus) serratus* (Theobald)	Terra-Firme	1.12
	Culex (Culex) coronator Dyar and Knab	Curió-Utinga	0.56
Pantoea sp.	*Coquillettidia (Rhynchotaenia) venezuelensis* (Theobald)	Terra-Firme	2.23
	Trichoprosopon (Trichoprosopon) spp.		
	Culex (Culex) quinquefasciatus Say	Cremação	0.56
		Outeiro	2.23
Proteus mirabilis	*Aedes (Stegomyia) aegypti* (Linnaeus)	Tapanã	0.56
Providencia rettgeri	*Culex (Culex) quinquefasciatus* Say	Cremação	1.12
		Jurunas	2.23
Providencia rustigianii	*Aedes (Stegomyia) aegypti* (Linnaeus)	Tapanã	0.56
Pseudomonas aeruginosa	*Aedes (Stegomyia) aegypti* (Linnaeus)	Tapanã	3.35
	Culex (Culex) quinquefasciatus Say		
Pseudomonas sp.	*Trichoprosopon (Trichoprosopon)* spp.	Terra-Firme	1.12
Staphylococcus sp.	*Culex (Culex)* spp.	Terra-Firme	6.70
	Coquillettidia (Rhynchotaenia) venezuelensis (Theobald)		
	Ochlerotatus (Ochlerotatus) serratus (Theobald)		
	Anopheles (Nyssorhynchus) triannulatus s.l. (Neiva and Pinto)	Curió-Utinga	1.12
		Nazaré	2.23
Stenotrophomonas maltophilia	*Culex (Culex)* spp.	Curió-Utinga	1.12
	Ochlerotatus (Ochlerotatus) serratus (Theobald)	Terra-Firme	1.12
Streptococcus mitis	*Ochlerotatus (Ochlerotatus) serratus* (Theobald)	Terra-Firme	3.35
Streptococcus pyogenes	*Coquillettidia (Rhynchotaenia) venezuelensis* (Theobald)	Terra-Firme	2.23
Streptococcus sp.	*Culex (Culex) quinquefasciatus* Say	Outeiro	0.56

three species and the other bacteria species were found in only one or two species of Culicidae. *E. cloacae* also occurred in the greatest number of collection points (five districts). *G. haemolysans*, *Phantoea* sp. and *Staphylococcus* sp. occurred in three districts, and the other bacteria species appeared in one to two neighborhoods. It was also observed that some bacteria species exhibited an elevated frequency in certain Culicidae species: *G. haemolysans* in *C. (C.) quinquefasciatus* (7.82% of all isolated and identified strains); *Staphylococcus* sp. and *Culex (Culex)* spp. (6.7%), *E. faecalis* in *C. (C.) quinquefasciatus* (6.14%), *B. Cereus* in *Culex (Culex)* spp. (5.59%); *G. haemolysans* in *A. (N.) aquasalis* (5.02%); *Bacillus* sp. in *Coquillettidia (R.) venezuelensis* (4.47%). All others had rates below 4%.

Figure 3 illustrates the number of bacteria species at each Culicidae collection point. The Curió-Utinga and Terra-Firme sampling points had the greatest number of bacteria species. The most frequently occurring bacteria in these points were *Bacillus cereus* (5.59% of all strains identified) and *Gemella haemolysans* (7.82% of all strains identified). Moreover, the Outeiro and Icoaraci collection points had only two bacteria species, with the *Pantoea* sp. (2.23% of all strains identified) dominant in Outeiro and *E. cloacae* (1.67% of total strains identified) in Icoaraci.

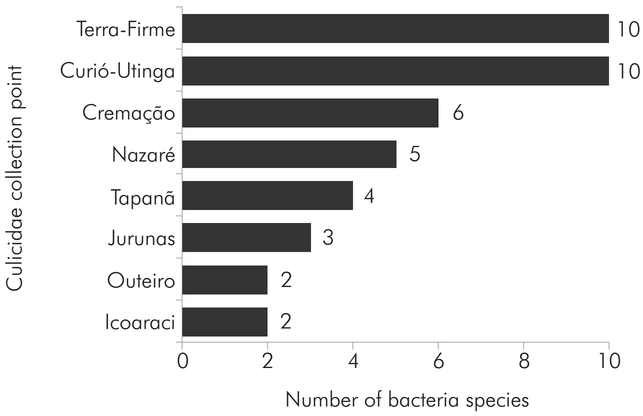

Figure 3 – Number of species identified in Culicidae in each collection point

Figure 4 shows the number of bacteria species found in each Culicidae species. *Culex quinquefasciatus*, *Coquillettidia venezuelensis* and *A. aegypti* were Culicidae species that contained the greatest number of bacteria species. There was no bacterial growth in culture media with *Psorophora ferox* and *Phoniomyia* spp. specimens.

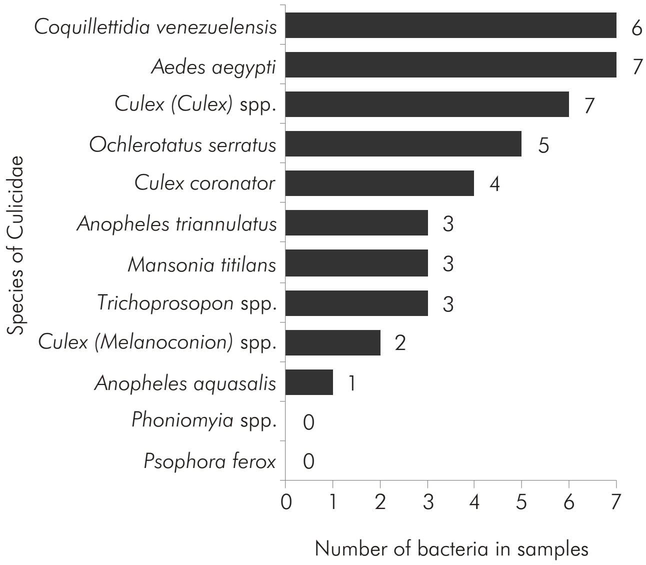

Figure 4 – Number of bacteria species in each species of Culicidae

DISCUSSION

The Culicidae are closely associated with human activity, which provides these species with artificial oviposition sites, and allows the maintenance of their populations. Urban centers favor denser, more disperse populations of mosquitoes, since the organized social space influences the interaction between the vectors, infectious agents, and humans. Among the species of collected mosquitoes, *C. quinquefasciatus* and *A. aegypti* were the species with the greatest ability to colonize these urban areas.

C. quinquefasciatus were collected in five of the eight defined collection points, confirming that it is a species with a strong ability to disperse. In a study performed in 1991[45], females in this species were initially marked and were recaptured 1 km from the release point. This factor is essential for determining the potential spread of this species, which can also be confirmed by the bacteria identified in the samples because the *Enterobacter amnigenus* species was isolated in districts located far from each other like Cremação and Icoaraci, and *Pantoea* sp. was isolated in samples from Cremação and Outeiro. These points are distant and have distinct environmental characteristics, but they presented the same vector and bacterial microbiota.

Among Culicidae, the *C. quinquefasciatus* species was the most abundant (85 specimens) and contained 8 different bacteria samples, with *Enterococcus faecalis* most frequently occurring (13 occurrences). Adult females of this species tend to feed on human blood[10], which allows their development in urban environments. In addition to being a nuisance to the communities in close proximity to their nests[48]. *C. quinquefasciatus* is a vector of several pathogens to humans[19]. Thus this species is characterized as being of great importance to public health, justifying its control policies in infested areas.

A. aquasalis is identified as an anthropophilic, zoophilic or, occasionally, eclectic vector. It is a major coastal malaria vector in several locations in Brazil and the Americas, and has shown highly varying feeding habits[18]. Forty two samples were collected during two collections in the neighborhood of Nazaré. *Gemella haemolysans* bacteria was the sole species identified in the samples.

The *A. aegypti* species was the third most frequently collected (40 specimen) during the study period. This species was encountered in the Jurunas and Tapanã neighborhoods and also in the administrative district of Icoaraci. In the samples, six different species were identified, including, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The *Aedes aegypti* mosquito is potentially the most critical health problem in Brazil today, considering its presence in all states and its transmission of the virus that causes dengue fever, hemorrhagic dengue fever, and yellow fever[6].

Coquillettidia venezuelensis, which was collected in the neighborhoods of Curió-Utinga and Terra-Firme, presented a large variety of bacterial samples (8), but has a decreased transmission potential because of its required forested habitat. According to Guimarães[23], in areas of the Serra do Mar in São Paulo, the *Coquillettidia* species require certain environmental factors for their life cycle, such as high rainfall and the presence of aquatic plants for their development. The work of Guimarães[23] confirms the results of this study, because the neighborhoods where *Coquillettidia* were collected had the ideal characteristics for their life cycle. Although this species possesses a wide variety of bacteria, it can only cause problems for people living in these ideal conditions.

Among the bacteria, *Gemella haemolysans* was the most frequently identified (14.51% of all identified strains). This species was present in samples of *A. aegypti*, *A. aquasalis*, *Trichoproson* spp. and *O. serratus*. This bacterium can normally be found in oral cavities, provoking gingivitis, or even meningitis, bronchitis and pneumonia[34,16]. Bacteria of the *Gemella* fgenus were the main finding in the Moreira[38] study, when isolating ants from hospitals in Rio de Janeiro.

The *Enterobacter cloacae* species occurred 22 times in 6 Culicidae samples and represented 12.28% of all strains identified. The only sample collection point where these bacteria were not found was in the neighborhood of Jurunas. This species is usually present in water, sewage, soil, and plants and is also part of the commensal enteric microbiota. It is thought not to cause diarrhea and is also associated with a variety of opportunistic infections that affect the urinary tract, respiratory tract, wounds and septicemia[28]. This species was also found in a study developed with cockroaches in Goiânia[44]. Gouveia et al[22] found a significant prevalence of *E. cloacae* in distinct populations of *Lutzomyia*, which is similar to the present study's findings for Culicidae.

Bacteria of the coagulase-negative *Staphylococcus* genus are widely distributed in the environment and are part of the nasal microbiota, but can also be found in the oral cavity[33]. All strains of staphylococci of the Culicidae tested negative for coagulase, excluding the possibility of *Staphylococcus aureus*, which is one of the most important of the genus because it is involved in several pathologies from food poisoning[33] to septicemia[16]. The results of a recent study developed by Costa[11] revealed that bacteria of the genus *Staphylococcus*, that tested negative for coagulase, were the main finding of the bacteriological research on ants in hospitals in Minas Gerais, illustrating the species' ability to be transported by mechanical vectors.

Enterococcus faecalis was isolated from *C. quinquefasciatus* in the collection points of Cremação and Jurunas and from *A. aegypti* in the Jurunas collection point. This species represented 8.93% of all isolated bacterial colonies and was most frequent in samples of *C. quinquefasciatus* from the Cremação collection point (6.14%). Enterococci are Gram-positive cocci that are usually found in pairs and short chains. They can be found in soil, food, water, animals, birds and insects. The main human reservoir of Enterococci is the gastrointestinal tract, but it can also be found less frequently in the oral cavity, gall bladder, vagina and male urethra[53]. In recent years, several studies were commissioned because *Enterococci* have become significant agents of human diseases primarily due to its resistance to antimicrobial agents[38].

Bacillus cereus and *Bacillus* sp. represented 5.59% and 7.26% of all bacteria strains identified, respectively. *B. cereus* was isolated from *C. coronator, C. (Culex)* spp. and *A. triannulatus* captured at the Curió-Utinga collection point. *Bacillus* sp. was isolated from *A. triannulatus* at the Nazaré sampling site and *C. venezuelensis* was isolated from sampling in the Curió-Utinga neighborhood. *B. cereus* is a Gram-positive bacterium found in soil. However, due to the resistance of its spores, the bacteria can be isolated from a variety of points and is widely distributed in nature. According to Mendes et al[35] its main anthropologic implication is in food contamination because it may cause deterioration of food in stock and diarrhea when it is consumed[39]. However, *Bacillus* sp. is usually associated with contamination of milk. According to Vittoril et al[52], thermal processing of milk is not able to destroy these bacteria.

Pantoea sp. was isolated from *C. venezuelensis*, *T. (Trichoprosopon)* spp. and *C. quinquefasciatus* collected at the Terra-Firme, Cremação and Outeiro collection points. The frequency of this species was 5.02% of all strains identified. *Pantoea* sp. are short Gram-negative bacilli and are usually isolated from plant surfaces, seeds, soil and water. They are opportunistic pathogens and therefore may be present in wounds, blood and human urine[26].

Gemella morbillorum was isolated from *C. (Culex)* spp. and *Coquillettidia venezuelensis* at the Terra-Firme collection point at a frequency of 3.91% of all strains identified. It is a commensal bacterium of the oropharynx, upper respiratory, urogenital and gastrointestinal tracts, although they rarely cause infections in humans. However, a growing number of infections in different locations have been reported[16,25,29]. Brain abscesses caused by this bacterium are extremely rare with only four cases described in scientific literature[29].

Pseudomonas aeruginosa was isolated from *A. aegypti* and *C. quinquefasciatus* from the Tapanã collection point with a frequency of 3.35%. This species is Gram-negative and is an extremely versatile bacteria that can be found in various environments, especially soil, water, plants and animals, and may cause opportunistic infections. In humans, *P. aeruginosa* causes infections in immuno-compromised individuals, such as AIDS and cancer patients, burn victims and those with cystic fibrosis[1]. *P. aeruginosa* is also commonly found in nosocomial infections since it is able to adhere to different materials, allowing it to contaminate catheters, ventilators, prosthetics and contact lenses. Because of the high resistance to antibiotics and the great amount of virulence factors, infections caused by this bacterium are difficult to control[4].

Providencia rettgeri and *Providencia rustigianii* Providencia rettgeri and Providencia rustigianii together showed a frequency of 3.91% of the all strains identified. They were isolated from *C. quinquefasciatus* captured in the Cremação and Jurunas collection points, respectively, and from *A. aegypti* that were captured at the collection point of Tapanã. The *Providencia* genus is currently composed of five species *P. alcalifaciens, P. stuartii, P. rettgeri, P. rustigianii* and *P. heimbachae*, of which the first four are recognized as human pathogens. These species are commonly associated with urinary tract infections in healthy communities and in patients with catheters. They may cause various opportunistic infections in hospitalized patients with burns, skin lesions, surgical wounds and septicemia[2].

The genus *Streptococcus* contains many species of Gram-positive cocci, facultative, commensal and pathogenic anaerobes that colonize the skin and mucous membranes of the respiratory tract, genitourinary and alimentary canals of humans and other mammals[32]. In this work, *Streptococcus mitis* and *Streptococcus pyogenes* were isolated and identified by species; a third strain could not be categorized to the species level. *Streptococcus mitis* was isolated from *Ochlerotatus serratus* collected from Terra-Firme at a frequency of 3.35% of the total of strains identified. This is a dominant species in mucous membranes and on tongues of humans[32]. *S. pyogenes* had a frequency of 2.23% and was isolated from *C. venezuelensis* captured at the Terra-Firme collection point. This species is also known as Group A beta-hemolytic streptococcus (GABHS). It is the main representative of the beta-hemolytic streptococci, which has shown a strong ability to adapt to a human host over time, acting as an important etiologic agent in a number of clinical manifestations, predominately in the oropharynx[32], as well as non-suppurative sequelae, which are characterized by rheumatic fever and glomerulonephritis.

Stenotrophomonas maltophilia was isolated from *Culex* spp. collected at the Curió-Utinga collection point and from *Ochlerotatus serratus* collected at Terra-Firme. It occurred at a frequency of 2.24% of all strains identified. It is a bacterium in the form of Gram-negative bacillus that can be found in a wide variety of environments and geographical regions, occupying different ecological niches and multiple sources of water. Other sources of isolation include soil, debris, raw milk, frozen fish, eggs and animal carcasses[46]. In hospital environments, this species has been isolated from tap water, sinks, respirators, suction catheters, blood pressure monitors, dialysis equipment and, occasionally, the hands of health care professionals[12]. Currently, *S. maltophilia* is considered an emerging pathogen because it occupies an important role in the setting of nosocomial infections, accounting for high morbidity because of its intrinsic resistance to most available antibiotics[12].

Morganella morganii was isolated from *O. serratus* and *C. coronator* that were collected at sampling sites of Terra Firme and Curió-Utinga, respectively. It presented a frequency of less than 2% of all isolated strains. This species is Gram-negative and occurs naturally in the soil and feces of animals and humans. Recent studies of Kara José et al[27] described *M. morganii* as a major contaminant of ophthalmic solutions, which can cause eye inflammation.

The species *Klebsiella oxytoca* and *Klebsiella pneumoniae* were isolated from *C. venezuelensis* and *A. aegypti*, respectively. Both had less than 2% frequency in Culicidae analyzed. However, these species are important because they cause severe infections and are resistant to several antibiotics. *K. pneumoniae* is a gram-negative bacillus of the family Enterobacteriaceae that is found in the upper respiratory, gastrointestinal and urinary tracts and can cause lobar pneumonia, urinary tract infection and septicemia. Several studies have tested the sensitivity of *K. pneumoniae* strains to antibiotics. Menezes et al[36] found that the drug Meropenem is a good choice for treating infections caused by this bacterium. Since *K. oxytoca* is more opportunistic, it may worsen conditions and cause bacteremia after invasive procedures are performed[7].

Cedecea neteri occurred in less than 2% of all identified strains, and was isolated from *Mansonia titillans* from the Curió-Utinga sampling point and *Culex quinquefasciatus* from the Cremação collection point. Enterobacteria of the genus *Cedecea* are characterized as short bacilli possessing biochemical reactions similar to those of the genus *Serratia*. Described in 1981, they have not had their pathogenic relevance well defined yet. The genus *Cedecea* includes the species *C. davisae*, *C. neteri* and *C. lapagei*, along with two species that are not yet named. This species has been isolated from humans in about 50% of the cases of respiratory tract infection. There are few reports of bacteremia in humans caused by *C. neter* given that the genus *Cedecea* is a rare opportunistic agent[39].

Proteus mirabilis represented less than 1% of the all strains identified and was isolated from *A. aegypti* captured at the Tapanã collection point. Although few strains were isolated in this study, *P. mirabilis* is one of the most clinically significant species as it accounts for 10% of uncomplicated urinary tract infections and is the fifth most common pathogen responsible for urinary tract infections in hospitals. This species may also cause infections of wounds and sepsis in hospitalized patients.

CONCLUSION

The results of this study evidence an important relationship between Culicidae and bacteria by which a diverse and dynamic natural reservoir is maintained for the colonization of humans and animals. Moreover, this relationship reveals the importance of ecological and epidemiological studies involving bacteria and their vectors.

In the past ten years, there has been an increase in scientific production on relationships between insects and bacteria. This work is still being carried out, and continued efforts are important for the advancement of knowledge and eventual consolidation of an emerging line of research.

This is the first study developed in South America that researches the transport of bacteria in insects of the Culicidae family, and thus serves as a basis for further research on the relationship between these two living beings that present medical and veterinary significance.

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Bactérias isoladas de culicídeos (Diptera: Nematocera) hematófagos em Belém, Pará, Brasil

RESUMO

As bactérias estão abundantemente distribuídas na natureza, participam da microbiota humana e animal, e algumas causam doenças. Têm a sua distribuição ampliada quando são veiculadas por algum vetor. Os dípteros da família Culicidae são vetores em epidemias de grande interesse para a saúde pública; no entanto, a associação entre bactérias e culicídeos foi pouco abordada. Para avançar conhecimento sobre esta temática, foi proposto isolar e identificar bactérias que estão sendo transportadas em culicídeos hematófagos em Belém, Pará. As coletas dos culicídeos foram realizadas com armadilha luminosa do tipo CDC, em oito pontos de coleta da área metropolitana de Belém, que apresentam características ambientais distintas. Foram coletados 296 exemplares de culicídeos, sendo que nove foram identificados até o nível de espécie e quatro até o subgênero. Destas amostras foi possível identificar 17 espécies de bactérias, outras sete somente foram identificadas até o gênero. *Culex quinquefasciatus* e *Anopholes aquasalis* foram os mais abundantes entre os culicídeos. As espécies de bactérias *Gemella haemolysans* e *Enterobacter cloacae* foram as mais abundantes nas amostras. Os pontos de coleta localizados nos bairros da Terra Firme e Curió Utinga foram os que apresentaram maior diversidade de espécies de culicídeos.

Palavras-chave: Bactérias; Culicidae; Transporte Biológico; Ecossistema Amazônico.

Bacterias aisladas de culícidos (Diptera: Nematocera) hematófagos en Belém, Pará, Brasil

RESUMEN

Las bacterias son abundantes en la naturaleza, participan de la flora y de la fauna animal, y algunas causan enfermedades. Tienen su distribución ampliada, cuando son transportadas por un vector. Los Diptera de la familia Culicidae, son vectores de epidemias de gran interés para la salud pública, sin embrago, la asociación entre las bacterias y los culícidos ha sido poco abordada. Para avanzar en el conocimiento sobre este tema, se propuso aislar e identificar las bacterias que son transportadas por los culícidos hematófagos en Belém, Pará. La captura de los culícidos se realizó con una trampa de luz de tipo CDC, en ocho puntos de colecta en el área metropolitana de Belém, que presentan distintas características ambientales. Fueron recogidas 296 muestras de culícidos, algunos de los cuales fueron identificados a nivel de especie (9) y otros para subgénero (4). De estas muestras se identificaron 17 especies de bacterias, otras siete fueron identificadas sólo a nivel de género. *Culex quinquefasciatus* y *Anopheles aquasalis* que fueron los más abundantes entre los culícidos. Las especies de bacterias *Gemella haemolysans* y *Enterobacter cloacae* fueron las más abundantes en las muestras. Los puntos de colectas se ubicaron en los barrios de Terra-Firme y Curió-Utinga, que fueron los que presentaron la mayor diversidad de especies de culícidos.

Palavras clave: Bactérias; Culicidae; Transporte Biológico; Ecosistema Amazônico.

REFERENCES

1 Ali NJ, Kessel D, Miller RF. Bronchopulmonary infection with *Pseudomonas aeruginosa* in patients infected with human immunodeficiency virus. Genitourin Med. 1995 Apr;71(2):73-7.

2 Almeida MTG, Bertelli ECP, Rossit ARB, Bertollo EMG, Martinez MB. Infecções hospitalares por *Stenotrophomonas maltophilia*: aspectos clínico-epidemiológicos, microbiológicos e de resistência antimicrobiana. Arq Cienc Saude [Internet]. 2005 jul-set [citado 2009 jan 13];12(3):141-5. Disponível em: http://www.cienciasdasaude.famerp.br/racs_ol/vol-12-3/04%20-%20ID129.pdf.

3 Alves LFA, Alves SB, Lopes J, Lopes RB. Avaliação de estirpes e de uma nova formulação granulada de *Bacillus sphaericus* Neide para o controle de mosquitos. Neotrop Entomol. 2006 jul-ago;35(4):493-9.

4 Arruda EAG. Infecção hospitalar por *Pseudomonas aeruginosa* multi-resistente: análise epidemiológica no HC-FMUSP. Rev Soc Bras Med Trop. 1998 set-out; 31(5):503-4.

5 Bobrowski VL, Fiuza LM, Pasquali G. Bodanese-Zanettini MH. Genes de *Bacillus thuringiensis*: uma estratégia para conferir resistência a insetos em plantas. Cienc Rural [Internet]. 2003 set-out [citado 2009 fev 20];33(5):843-50. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782003000500008&lng=en&nrm=iso& tlng=pt.

6 Braga IA, Valle D. *Aedes aegypti*: inseticidas, mecanismos de ação e resistência. Epidemiol Serv Saude [Internet]. 2007 dez [citado 2009 fev 20];16(4):279-93. Disponível em: http://scielo.iec.pa.gov.br/scielo.php?script=sci_abstract&pid=S1679-49742007000400006&lng=pt&nrm=iso&tlng=pt.

7 Campos GMR, Herani Filho B, Pereira CAP, Machado AMO, Baretta MCC. Bacteremia após a colangiopancreatografia retrógrada endoscópica, com e sem procedimento terapêutico: freqüência, fatores associados e significado clínico. Rev Assoc Med Bras [Internet]. 1997 out-dez [citado 2009 jan 21];43(4):326-34. Disponível em: http://www.scielo.br/scielo.php?pid=S0104-42301997000400009&script=sci_arttext.

8 Carvalho CJB, Couri MS, Toma R, Rafael JA, Harada AY, Bonatto SR, et al. Principais coleções brasileiras de Diptera: histórico e situação atual. In: Costa C, Vanin SA, Lobo JM, Melic A. Proyecto de Red Iberoamericana de Biogeograía y Entomología Sistemática (PrIBES). Zaragoza: Sociedade Entomológica Aragonesa; 2002. Vol. 2, p. 37-52.

9 Cerqueira NL. Distribuição geográfica dos mosquitos da Amazônia (Diptera: Culicidae: Culicinae). Rev Bras Entomol. 1961;10:111-68.

10 Consoli RAGB, Lourenço-de-Oliveira R. Principais mosquitos de importância sanitária no Brasil. Rio de Janeiro: FIOCRUZ; 1994. 225 p.

11 Costa SB, Pelli A, Carvalho G, Oliveira AG, Silva PR, Teixeira MM, et al. Formigas como vetores mecânicos de microorganismos no Hospital Escola da Universidade Federal do Triângulo Mineiro. Rev Soc Bras Med Trop. 2006 nov-dez;39(6):527-9.

12 Denton M, Kerr KG. Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. Clin Microbiol Rev. 1998 Jan;11(1):57-80.

13 Dequech STB, Fiuza LM, Silva RFP, Zumba ARC. Histopatologia de lagartas de *Spodoptera frugiperda* (Lep., Noctuidae) infectadas por *Bacillus thuringiensis aizawai* e com ovos de *Campoletis flavicincta* (Hym., Ichneumonidae). Cienc Rural. 2007 jan-fev;37(1): 273-6.

14 Dimopoulos G, Richman A, Müller HM, Kafatos FC. Molecular immune responses of the mosquito *Anopheles gambiae* to bacteria and malaria parasites. Proc Natl Acad Sci U S A. 1997 Oct;94(21):11508-13.

15 Edwards PR, Ewing WH. Identification of *Enterobacteriaceae*. 4th ed. New York: Elsevier Science Publishing; 1986. 362 p.

16 Eisenhut M, Jones C, Hughes D, Herrington S, Kokai G. Acute renal failure associated with *Gemella haemolysans* pneumonia. Pediatr Nephrol. 2004 Apr; 19(4):448-50.

17 Faran ME, Linthicum KJ. A handbook of the Amazonian species of *Anopheles* (*Nyssorhynchus*) (Diptera: Culicidae). Mosq Syst. 1981;13:1-81.

18 Flores-Mendoza C, Cunha RA, Rocha DS, Lourenço-de-Oliveira R. Determinação das fontes alimentares de *Anopheles aquasalis* (Diptera: Culicidae) no Estado do Rio de Janeiro, Brasil, pelo teste de precipitina. Rev Saude Publica. 1996 abr;30(2):129-34.

19 Forattini OP. Culicidologia médica: identificação, biologia, epidemiologia. São Paulo: EDUSP; 2002. Vol. 2, 860 p.

20 Gonzalez-Ceron L, Santillan F, Rodriguez MH, Mendez D, Hernandez-Avila JE. Bacteria in midguts of field-collected *Anopheles albimanus* block *Plasmodium vivax* sporogonic development. J Med Entomol. 2003 May;40(3):371-4.

21 Gorham JR, Stojanovich CJ, Scott HG. Clave ilustrada para los mosquitos anofelinos de Sudamerica Oriental. Atlanta: Public Health Service; 1967. 64 p.

22 Gouveia C, Asensi MD, Zahner V, Rangel EF, Oliveira SMP. Study on the bacterial midgut microbiota associated to different Brazilian populations of *Lutzomyia longipalpis* (Lutz & Neiva) (Diptera: Psychodidae). Neotrop Entomol. 2008 Sep-Oct;37(5):597-601.

23 Guimarães AE, Gentile C, Lopes CM, Mello RP. Ecology of mosquitoes (Diptera: Culicidae) in areas of Serra do Mar State Park, State of São Paulo, Brazil. II - Habitat distribution. Mem Inst Oswaldo Cruz [Internet]. 2000 Jan-Feb [citado 2009 mar 6];95(1):17-28. Disponível em: http://www.scielo.br/scielo.php?pid=S0074-02762000000100002script=sci_art text.

24 Gusmão DS, Santos AV, Marini DC, Russo ES, Peixoto AMD, Bacci Júnior M, et al. First isolation of microorganisms from the gut diverticulum of *Aedes aegypti* (Diptera: Culicidae): new perspectives for an insect-bacteria association. Mem Inst Oswaldo Cruz. 2007 Dec;102(8):919-24.

25 Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. Bergey's manual of determinative bacteriology. 9th ed. Baltimore: Williams & Wilkins; 1994. 787 p.

26 Hörner R, Liscano MGH, Maraschin MM, Salla A, Meneghetti B, Dal Forno NL, et al. Suscetibilidade antimicrobiana entre amostras de *Enterococcus* isoladas no Hospital Universitário de Santa Maria. J Bras Patol Med Lab. 2005 dez;41(6):391-5.

27 Kara José AC, Castelo Branco B, Ohkawara LE, Yu MCZ, Lima ALH. Uso ocular de água boricada: condições de manuseio e ocorrência de contaminação. Arq Bras Oftalmol. 2007 mar-abr;70(2):201-7.

28 Koneman EW, Allen SD, Janda WM, Schreckenberger PC. Diagnóstico microbiológico. 5. ed. Rio de Janeiro: MEDSI; 2001. 1465 p.

29 Lopes A, Providencia R, Pais RP, Frade MJ, Chaddad Neto F, Oliveira E. Cerebellar abscess by *Gemella morbillorum* in a patient with inter-atrial communication. Arq Neuropsiquiatr. 2007 Dec;65(4 A):1022-5.

30 Luz C, Sebba GJ, Silva NR, Silva HHG, Monerat R. Prospecção de bactérias entomopatogênicas em solos de cerrado para controle biológico de mosquitos. Inf Epidemiol Sus. [Internet]. 2001 [citado 2009 out 17];10 Suppl 1:49-50. Disponível em: http://scielo.iec.pa.gov.br/scielo.php?script=sci_arttext&pid=S0104-16732001000500015&lng=pt&nrm=iso.

31 Luz-Alves WC, Gorayeb IS, Silva JCL, Loureiro ECB. Bactérias transportadas em mutucas (Diptera: Tabanidae) no nordeste do Pará, Brasil. Bol Mus Para Emilio Goeldi Cienc Nat. 2007 abr-jul;2(3):11-20.

32 Maciel A, Aca IS, Lopes ACS, Malagueño E, Sekiguchi T, Andrade GP. Portadores assintomáticos de infecções por *Streptococcus pyogenes* em duas escolas públicas na cidade do Recife, Pernambuco. Rev Bras Saude Mater Infant. 2003 jun;3(2):175-80.

33 Martins CAP, Koga-Ito CY, Jorge AOC. Presence of *Staphylococcus* spp. and *Candida* spp. in the human oral cavity. Braz J Microbiol. 2002 Jul-Set;33(3):236-40.

34 May T, Amiel C, Lion C, Weber M, Gerard A, Canton P. Meningitis due to *Gemella haemolysans*. Eur J Clin Microbiol Infect Dis. 1993 Aug;12(8):644-5.

35 Mendes RA, Azeredo RMC, Coelho AIM, Oliveira SS, Coelho MSL. Contaminação ambiental por *Bacillus cereus* em unidade de alimentação e nutrição. Rev Nutr. 2004 abr-jun;17(2):255-61.

36 Menezes EA, Nascimento KM, Soares KP, Amorim LN, Lima Neto JG, Cunha FA. Avaliação da atividade in *vitro* do meropenem contra cepas de *Klebsiella pneumoniae* produtoras de betalactamases de espectro expandido isoladas na cidade de Fortaleza, Ceará. Rev Soc Bras Med Trop. 2007 mai-jun;40(3): 349-50.

37 Moreira DDO, Morais V, Vieira-da-Motta O, Campos-Farinha AEC, Tonhasca Junior A. Ants as carriers of antibiotic-resistant bacteria in hospitals. Neotrop Entomol. 2005 Nov-Dec;34(6):999-1006.

38 Moreira M, Medeiros ACC, Pignatari SB, Wey SB, Cardo DM. Efeito da infecção hospitalar da corrente sanguínea por *Staphylococcus aureus* resistente à oxacilina sobre a letalidade e o tempo de hospitalização. Rev Ass Med Bras. 1998;44(4): 263-8.

39 Murray PR, Pfaller MA, Kobayashi GS, Rosenthal KS. Microbiologia médica. 4. ed. Rio de Janeiro: Guanabara Koogan; 2004. 766 p.

40 Oliveira SMP, Morais BA, Gonçalves CA, Giordano-Dias CM, Vilela ML, Brazil RP, et al. Microbiota do trato digestivo de fêmeas de *Lutzomyia longipalpis* (Lutz & Neiva, 1912) (Diptera: Psychodidae) provenientes de colônia alimentadas com sangue e com sangue e sacarose. Cad Saude Publica [Internet]. 2001 [citado 2009 mar 15];17(1):229-32. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0102-311X2001000100024&lng=en&nrm=iso&tlng=pt.

41 Oliveira VC, D'Almeida JM, Abalem IV, Mandarino JR, Solari CA. Enterobactérias associadas a adultos de *Musca domestica* (Linnaeus, 1758) (Diptera: Muscidae) e *Chrysomya megacephala* (Fabricius, 1754) (Diptera: Calliphoridae) no Jardim Zoológico, Rio de Janeiro. Arq Bras Med Vet Zootec. 2006 ago;58(4):556-61.

42 Papavero N, editor. A catalogue of Diptera of the Americas South of United States. São Paulo: USP; 1967.

43 Polanczyk RA, Garcia MO, Alves SB. Potencial de *Bacillus thuringiensis israelensis* Berliner no controle de *Aedes aegypti*. Rev Saude Publica. 2003;37(6): 813-6.

44 Prado MA, Pimenta FC, Hayashid M, Souza PR, Pereira MS, Gir E. Enterobactérias isoladas de baratas (*Periplaneta americana*) capturadas em um hospital brasileiro. Rev Panam Salud Publica. 2002 fev;11(2):93-8.

45 Reisen WK, Milby MM, Meyer RP, Pfutner AR, Spoehel J, Hazelriqq JE, Webb JP Jr. Mark-release-recapture studies with *Culex* mosquitoes (Diptera: Culicidae) in southern California. J Med Entomol. 1991 May;28(3): 357-71.

46 Segabinazi SD. Presença de bactérias da família *Enterobacteriaceae* nas superfícies externa e interna de *Alphitobius diaperinus* (panzer) oriundos de granjas avícolas dos estados do Rio Grande do Sul e Santa Catarina [dissertação]. Santa Maria (RS): Universidade Federal de Santa Maria; 2004. 105 p.

47 Sudia WD, Chamberlain RW. Battery operated light trap and improved model. Mosq News. 1962;22(2):126-9.

48 Taipes-Lagos CB, Natal D. Abundância de culicídeos em área metropolitana preservada e suas implicações epidemiológicas. Rev Saude Publica. 2003;37(3):275-9.

49 Teixeira MLF, Franco AA. Susceptibilidade de larvas de *Cerotoma arcuata* Olivier (Coleoptera: Chrysomelidae) a *Beauveria bassiana* (Bals.). Vuillemin, *Metarhizium anisopliae* (Metsch.) Sorokin e *Bacillus thuringiensis* Berliner. Cienc Rural [Internet]. 2007 jan-fev [citado 2009 jun 3];37(1):19-25. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782007000100004 lng=en&nrm=iso&tlng=pt.

50 Valicente FH, Barreto MR, Vasconcelos MJV, Figueiredo JEF, Paiva E. Identificação através de PCR dos genes *CryI* de cepas de *Bacillus thuringiensis* Berliner eficientes contra a lagarta do cartucho, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). An Soc Entomol Bras [Internet]. 2000 mar [citado jun 19];29(1):147-53. Disponível em: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0301-80592000000100018&lng=en&nrm=iso&tlng=pt.

51 Vieira PCB. Métodos de coletas de mosquitos (Diptera: Culicidae) alternativos ao de atração humana direta [dissertação]. Belém: Universidade Federal do Pará, Programa de Pós-graduação em Zoologia; 2007. 71 p.

52 Vittoril J, Schocken-Iturrino RP, Poiatti ML, Pigatto CP, Chioda TP, Ribeiro CAM. Qualidade microbiológica de leite UHT caprino: pesquisa de bactérias dos gêneros *Staphylococcus*, *Bacillus* e *Clostridium*. Cienc Rural. 2008 mai-jun;38(3): 761-5.

53 Xavier CAC, Oporto CFO, Silva MP, Silveira IA, Abrantes MR. Prevalência de *Staphylococcus aureus* em manipuladores de alimentos das creches municipais da cidade do Natal/RN. Rev Bras Anal Clin. 2007;39(3):165-8.

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