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, 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.

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 endemicism, 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 and collected 218 species, 152 of which were in the State of Pará. 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. Studies performed with mosquitoes bred in insectariums in Mexico and Brazil 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, while others are being tested as an alternative to controlling...
mosquito populations that cause epidemics. Some other studies are evaluating the association between bacteria with mechanical vectors and the possibility of transmission by various insects. In a 2007 study, 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 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, 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: Forantini, Gorham et al. and Faran and Finthicum.

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% CO2, 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. 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. 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 (Janthinosa) 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 (Janthinosa) ferox and Phoniomyia spp. showed no bacterial 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 species at the species level: Coquillettidia venezuelensis, Ochlerotatus serratus and Psorophora ferox. The subgenus Culex (Culex) spp., Trichoprosopon (Trichoprosopon) spp., and the Phoniomyia spp. 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.

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 Phaotes sp. were found in

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**Figure 1** – Culicidae species with respective numbers of collected specimens

**Figure 2** – Number of occurrences of bacteria species in samples

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

<table>
<thead>
<tr>
<th>Species of bacteria</th>
<th>Species of Culicidae</th>
<th>Collection locality</th>
<th>f (%) of bacteria</th>
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</thead>
<tbody>
<tr>
<td>Bacillus cereus</td>
<td>Culex (Culex) spp.</td>
<td>Curió-Utinga</td>
<td>5.59</td>
</tr>
<tr>
<td></td>
<td>Culex (Culex) coronator Dyar and Knab</td>
<td>Curió-Utinga</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>Anopheles (Nyssorhynchus) triannulatus s.l. (Neiva and Pinto)</td>
<td>Cremação</td>
<td>0.56</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>Anopheles (Nyssorhynchus) triannulatus s.l. (Neiva and Pinto)</td>
<td>Nazaré</td>
<td>2.79</td>
</tr>
<tr>
<td></td>
<td>Cedecea neteri</td>
<td>Curió-Utinga</td>
<td>2.23</td>
</tr>
<tr>
<td></td>
<td>Culex (Culex) quinquefasciatus Say</td>
<td>Cremação</td>
<td>1.67</td>
</tr>
<tr>
<td>Enterobacter amnigenus</td>
<td>Culex (Culex) quinquefasciatus Say</td>
<td>Nazaré</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>Culex (Culex) coronator Dyar and Knab</td>
<td>Icoaraci</td>
<td>1.67</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>Mansonia (Mansonia) triannulatus (Walker)</td>
<td>Curió-Utinga</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>Culex (Culex) quinquefasciatus Say</td>
<td>Cremação</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td>Cellulomonas sp.</td>
<td>Curió-Utinga</td>
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<td>Culex (Culex) spp.</td>
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<td>2.79</td>
</tr>
<tr>
<td></td>
<td>Culex (Melanocion) spp.</td>
<td>Icoaraci</td>
<td>1.12</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>Culex (Culex) quinquefasciatus Say</td>
<td>Nazaré</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>Aedes (Stegomyia) aegypti (Linnaeus)</td>
<td>Icoaraci</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>Gemella haemolysans</td>
<td>Ochlerotatus (Ochlerotatus) serratus (Theobald)</td>
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<td></td>
<td>Trichoprasapao (Trichoprasapao) spp</td>
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<tr>
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<td>Anopheles (Nyssorhynchus) aquasalis Curry</td>
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<td>Aedes (Stegomyia) aegypti (Linnaeus)</td>
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<td>Gemella morbillorum</td>
<td>Aedes (Stegomyia) aegypti (Linnaeus)</td>
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<td>Culex (Culex) spp.</td>
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<td>Klebsiella oxytoca</td>
<td>Coquilletidia (Rhynchotaenia) venezuelensis (Theobald)</td>
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<td>Klebsiella pneumoniae</td>
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<td>Morganella morganii</td>
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<td>Pantoea sp.</td>
<td>Coquilletidia (Rhynchotaenia) venezuelensis (Theobald)</td>
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<tr>
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<td>Proteus mirabilis</td>
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<td>Tapanã</td>
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<td>Providencia rettgeri</td>
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<td>Cremação</td>
<td>1.12</td>
</tr>
<tr>
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<td>Aedes (Stegomyia) aegypti (Linnaeus)</td>
<td>Juunas</td>
<td>2.23</td>
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<tr>
<td>Providencia rustigianii</td>
<td>Aedes (Stegomyia) aegypti (Linnaeus)</td>
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<td>Pseudomonas aeruginosa</td>
<td>Aedes (Stegomyia) aegypti (Linnaeus)</td>
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<td>Culex (Culex) quinquefasciatus Say</td>
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<td>Pseudomonas sp.</td>
<td>Trichoprasapao (Trichoprasapao) spp.</td>
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</tr>
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<td>Staphylococcus sp.</td>
<td>Culex (Culex) spp.</td>
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<td>Ochlerotatus (Ochlerotatus) serratus (Theobald)</td>
<td>Nazaré</td>
<td>2.23</td>
</tr>
<tr>
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<td>Anopheles (Nyssorhynchus) triannulatus s.l. (Neiva and Pinto)</td>
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<td>Stenotrophomonas maltophilia</td>
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<tr>
<td>Streptococcus millis</td>
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<td>Streptococcus pyogenes</td>
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<tr>
<td>Streptococcus sp.</td>
<td>Culex (Culex) quinquefasciatus Say</td>
<td>Outeiro</td>
<td>0.56</td>
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</table>
three species and the other bacteria species were found in only one or two species of Culicidae. *E. cloaca* also occurred in the greatest number of collection points (five districts). *G. haemolysans, Phantocea* 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 Curíó-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. cloaca* (1.67% of total strains identified) in Icoaraci.

![Figure 3](image)

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.

**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.
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.

Coquillettidia venezuelensis, which was collected in the neighborhoods of Curitiba-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, 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 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 haemolyans was the most frequently identified (14.51% of all identified strains). This species was present in samples of A. aegypti, A. aquasalis, Trichoprosop spp. and O. serratus. This bacterium can normally be found in oral cavities, provoking gingivitis, or even meningitis, bronchitis and pneumonia. Bacteria of the Gemella genus were the main finding in the Moreira 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. This species was also found in a study developed with cockroaches in Goiânia. Gouveia et al. 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. 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 to septicemia. The results of a recent study developed by Costa 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 Cremáçao 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 Cremáçao 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. In recent years, several studies were commissioned because Enterococci have become significant agents of human diseases primarily due to its resistance to antimicrobial agents.

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 Curitiba-Utinga collection point. Bacillus sp. was isolated from A. triannulatus at the Nazaré sampling site and C. venezuelensis was isolated from sampling in the Curitiba-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., its main anthropologic implication is in food contamination because it may cause deterioration of food in stock and diarrhea when it is consumed. However, Bacillus sp. is usually associated with contamination of milk. According to Vittori et al., 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, Cremáçao 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.

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 has been reported. Brain abscesses caused by this bacterium are extremely rare with only four cases described in scientific literature.

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. 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.

*Providencia rettgeri* and *Providencia rustigianii* showed a frequency of 3.91% of the all strains identified. They were isolated from *C. quinquefasciatus* captured in the Criação 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.

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. 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. *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 streptococci (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, 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. 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.

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.

*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. 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 septicaemia. Several studies have tested the sensitivity of *K. pneumoniae* strains to antibiotics. Menezes et al. 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.

*Cedecea neteri* occurred in less than 2% of all identified strains, and was isolated from *Mansonina stitillans* from the Curió-Utinga sampling point and *Culex quinquefasciatus* from the Criação 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. daviseae*, *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. neteri* given that the genus *Cedecea* is a rare opportunistic agent.

*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 dipteros 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 hematofágos 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 ao 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 Anopheles 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; Ecosistema 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 embargo, 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 hematofágos 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. Foron 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


