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Assessment of ants as bacterial vector in houses

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Studies of arthropods have fundamental importance to identifying both ant species and microorganisms they carry. The objective of this study was to identify ant morph species found in residences, characterize the bacteria associated with the ants, assess bacterial resistance to antibiotics and analyze the plasmid profiles of the microorganisms found. The ant collections were carried out in the kitchens of 50 residences in the daytime. The bacteria were quantified and the samples were confirmed for the presence of plasmids. The data demonstrate that *Pheidole* sp. (74%) was the most frequent ant among those isolated; the microbiology analysis showed that the genus *Sthaphyloccocus* sp. (90%) was the most prevalent bacterial genus found on ants. This research presented a low frequency of *Klebsiella* sp. (2%), *Enterococcus* sp. (2%) and *Vibrio cholera* (2%). The ants classified as *Pheidole* sp. and *Paratrechina* sp. showed greater presence of microorganisms in the ants' cuticule, and *Pheidole* sp. was prevalent in relation to other ant species. Resistance was found to the antibiotics ampicillin, erythromycin and penicillin. Since there was no plasmid in the samples, it can be suggested that this tolerance is of chromosomal bacterial origin.

Key words: Antimicrobial, ants, contamination vector, resistance.

INTRODUCTION

Of Brazil's 2,000 ant species, 20 to 30 are considered urban nuisances (Campos-Farinha, 2005). When humans seek comfort, they favor the survival of the urban ants offering food, shelter and moisture (Bueno et al., 1999). Most urban ants are omnivorous and present great mobility while looking for food, crossing garbage bins, drains and home environments, promoting contamination of their tegument while enlarging their potential vectorial (Thyssen et al., 2004). While colonizing domestic environments, these arthropods can damage electric appliances and cause contamination and disturbances for humans (Rodovalho et al., 2007).

Ants have several mutualistic and parasitic relationships with fungus, bacteria, animals and plants (Boursaux-Eude and Gross, 2000). Several studies conducted in hospitals have proven fungus and bacteria presence on the ants' tegument (Pesquero et al., 2008). As they are antibiotic-resistant microorganisms, these insects act like mechanical disease vectors, contributing to nosocomial infection (Moreira et al., 2005). However, some micro-organisms are known to be bacteria carried by ants in residences and food establishments (Zarzuela et al.,

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Table 1. Kind of bacteria have been reported in ant morphospecies.

Ant	Bacteria
Paratrechina sp.	*Streptococcus* sp., *Staphylococcus* sp., *Salmonella* sp., *Vibrio* sp., *Vibrio colerae*, Bacillus sp., Micrococcus sp. and Enterococcus sp.
Pheidole sp.	*Staphyloccus* sp., *Bacillus* sp., *Streptococcus* sp., *Salmonella* sp., *Micrococcus* sp., *Klebsiella* sp., *Vibrio* sp., *E. coli*., *Neisseria* sp. and *Enterobacteria* sp.
Brachymyrmex sp.	*Staphyloccus* sp., *Bacillus* sp., *Neisseria* sp. and *Vibrio* sp.
Dorymyrmex sp.	*Staphyloccus* sp., *Bacillus* sp. and *Vibrio* sp.
Tapinoma melanocephalus sp.	*Staphyloccus* sp., *Bacillus* sp. and *Enterobacteria* sp.
Tetramorium sp.	*Staphyloccus* sp., *Bacillus* sp. and *Salmonella* sp.

2004).

Diseases transmitted by foods (DTA) present a public health problem all over the world, being responsible for high economic and social costs (Welker et al., 2010). DTA thus leads to high mortality and morbidity rates (Martins et al., 2007). Bacteria function has been proven in food contamination cases (Andrade et al., 2003), showing that the microorganisms can be transported by ants.

Antibiotic-resistant bacteria have been studied frequently in urban ants (Kumar, 2012). The indiscriminate use of antibiotics by humans and for animal raising in the 1970's significantly increased the number and types of microorganisms resistant to these drugs (WHO, 2011). This higher resistance to antibiotics constitutes a global concern (van der Donk et al., 2012). Resistance is considered a natural phenomenon and resistance mechanisms are preexistent or naturally modified, both in gene transfers and in changes in bacterial chromosomes or plasmids (García et al., 2003).

The objective of the present work was to identify ant morph species found in residences. These were characterized as bacteria associated with ants. Bacterial resistance to antibiotics was measured and the microorganisms' plasmidial profiles were analyzed.

MATERIALS AND METHODS

Study location

The ant collections were done in the city of Morrinhos, Goiás, Brazil (17° 30' 05' a 18° 06' 11'' S e 48° 48' 49'' a 49° 27' 42'' W) in 50 residences in 20 urban neighborhoods.

Data collection: Mimercofauna

The ants were collected in kitchens in the morning using sugary bait (honey) and protein bait (sausage). The bait was left for an hour, which is approximately the period in which ant trails form. The ants were collected and stored in 70% alcohol with information about the place, date and time of collection.

Data collection: Microbiological

For microbiological analysis, the insects were collected using sterilized tweezers and cotton before coming into contact with the bait and then stored in new plastic bags, which were labeled and kept in a thermal box at approximately 10°C. After collection, the materials were taken to the laboratory for bacteria cultivation. An ant specimen was immersed in peptone water for 15 min, maintaining the nutritive medium at 37°C in a bacteriological incubator for 24 h.

Ant morphospecies identification

The collected specimens were identified by genus and morphospecies and, when possible, by species, using a dichotomous key for genus (Fernández, 2003) (Table 1).

Microbiological analyses

The number of microorganisms was determined by the serial dilution method (undiluted sample and serial dilutions of 10-1, 10-2 and 10-3) in "Plate Coute Agar" (PCA). For bacterial identification, Gram staining was done and the following biochemical tests were performed: catalase, motility, glucose, methyl red, Vougues Proskauer, phenylalanine deaminase, indole, sulfuric acid, urease and Simmons citrate agar. The following enriched mediums were used for identification: MacConkey agar, mannitol salt agar, bright green and EC broth. The results were interpreted according to Bergey's Manual of Systematic Bacteriology.

Antibiotic resistance analysis

The bacteria samples were seeded by scattering them in a petri dish containing Mueller Hinton agar and the inhibition zone of each paper disc was measured according to the Bauer-Kirby disc diffusion method. An antibiogram was performed to check for resistance to penicillin (10 µg), ampicillin (10 µg), erythromycin (15 µg) and gentamicin (10 µg), which are first- to third-generation antibiotics. The results were classified as sensitive, intermediate and resistant to the antibiotics tested, following the norms established by the National Committee for Clinical Laboratory Standards.

Data analysis

The antibiogram results were analyzed using the χ^2 test according

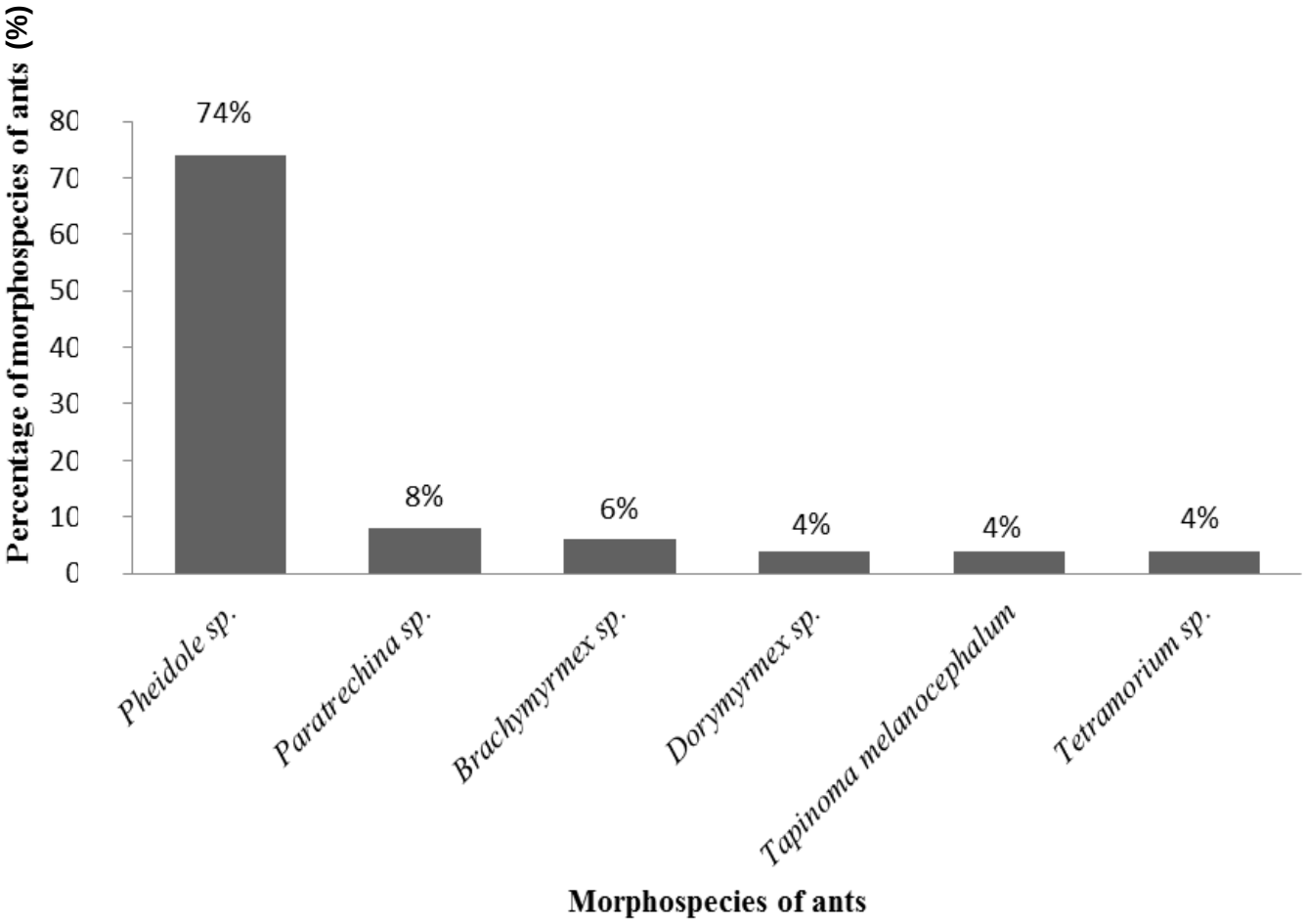

Figure 1. Percentage of ant morphospecies found in residence kitchens.

to the Systat 13 program.

Plasmid extraction

The presence of plasmid DNA was checked with the Pharmacia® FLEXIPREP extraction kit. The procedure was performed according to Sambrook, Fritsh and Maniats (1989). The sample was spiked in peptone water and incubated at 37°C for 24 h. An aliquot of 1.5ml was centrifuged at 5,000xg in eppendorf tubes for 30 s. This process was repeated three times. The sediment was resuspended in 200 µL of solution I and then homogenized. Subsequently, 200 µL of solution II was added and the material was mixed by inverting the tube for 5 min. After that, 200 µL of solution III was added and gently homogenized by inversion for 5 min and centrifuged at 5,000 xg for 5 min (according to the manufacturer's instructions). The sediment corresponding to the chromosomal DNA was discarded and the supernatant containing plasmid DNA was transferred to another tube. The DNA was precipitated by adding 420 µL of isopropanol and, after being homogenized, was placed to rest at room temperature for 10 min. It was then immediately centrifuged at 5,000 × *g* for 10 min. The supernatant was discarded and the precipitate was left to dry at room temperature. The precipitate was then resuspended in 50 µL of Milli Q water, homogenized and viewed in 1% (w/v) agarose gel with ethidium bromide and analyzed using electrophoresis.

RESULTS AND DISCUSSION

The ants classified as *Pheidole* sp. (74%) constituted the most frequent morph species found. *Dorymyrmex* sp. (4%), *Tapinoma melanocephalum* (4%), and *Tetramorium* sp. (4%), were the least frequent species found (Figure 1). Six ant morph species were found. This can be explained by the fact that these houses do not have the resources necessary for many arthropods to survive (Iop et al., 2009). The ant groups found in this study are common in urban environments (Lutinski et al., 2013). According to Wilson (2003), the genus *Pheidole* is abundant in neotropical regions, its dominance is due to its ability to communication and increased recruitment and aggressiveness in defense of food resources.

When ants and isolated bacteria were correlated, it was observed that the 50 samples collected were contaminated. Due to the *Pheidole* sp. species' abundance, it is responsible for the highest contamination rate. Ten bacterial genus were isolated from *Pheidole* sp. (Figure 2), followed by seven *Paratrechina* sp. isolated genus, and adding the 12 different bacterial types found

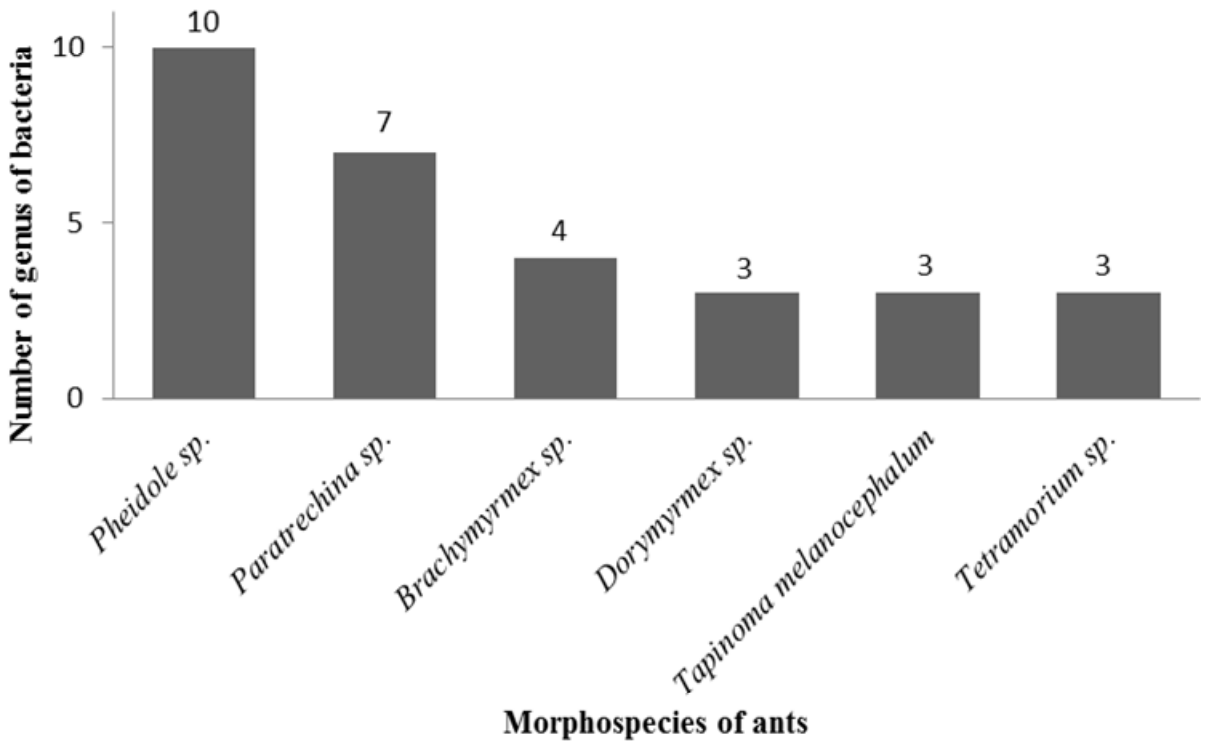

Figure 2. Relative value of bacterial genus isolated per ant.

in the ants.

The quantification was done using the most probable number methodology. The samples were immersed in "Plate Count agar (PCA) and 85.5% of the samples showed CFU\mL (colony forming units) countless count. Residences that showed smaller CFU\mL had been cleaned minutes before collection. It was observed that the houses that were cleaned before the ants were collected showed lower bacterial colonization, corroborating data showing the bactericidal effect of household disinfectants (INMETRO, 2008). This therefore demonstrates that contamination risk due to bacteria can be reduced by using hygenic practices. After the quantification was carried out, the identification of bacteria isolated on the ant tegument was performed. *Staphylococcus* sp. (90%), was the most common bacteria isolated and the *Klebsiella* sp., *Enterococcus* sp. and *Vibrio cholerae* families were the rarest, all having a 2% occurrence (Figure 3). Most of the ants hosted more than one bacterial genus.

Frequency of the *Staphylococcus* genus (90%), can lead to high levels of contamination and cause diseases related to the presence of domestic animals such as dogs and cats that harbor this bacterium in their fur (Hirsh and Zee, 2003). *Staphylococcus* sp. can also contaminate food scraps, causing several food-borne infections (Szweda et al., 2012). This bacteria genus is considered antimicrobial-resistant, being one of the effects of indiscriminate antibiotics use, both by humans and for domesticated animals (Soares et al., 2008).

The Table 2 presents the antibiogram results and demonstrates that all microorganisms tested were gentamicin-susceptible. The results for penicillin, were resistant (54%) and intermediate (44%) (χ^2 = 0. 32, p = 0. 57). Only 2% of the isolated bacteria showed sensitivity. For ampicillin, 74% of the samples tested showed resistance (χ^2 = 35. 65, p < 0. 0001) and erythromycin resistance was 84% (χ^2 = 55. 70, p < 0. 00001) samples. It is important note that the resistances detected in this study involve current antibiotics in clinical therapy. A resistance profile has been observed in household insects (Zarzuela et al., 2004), demonstrating the need for conscious antibiotics use.

The 50 samples were analyzed and no hosted plasmids were found. Any microorganism-hosted plasmid can suggest that antibiotics resistance is due to chromosomal DNA since changes in chromosomal DNA allow bacteria to survive in unfavorable conditions and multiply in addition to conferring antibiotic tolerance (Aly et al., 2012).

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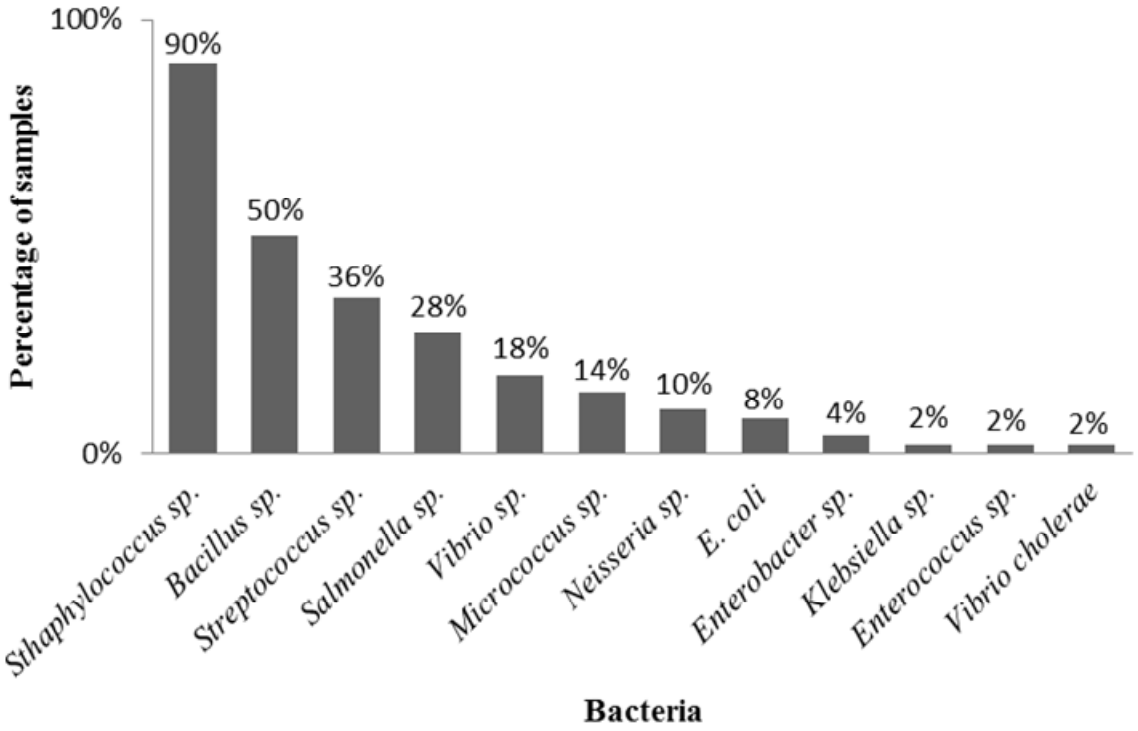

Figure 3. Percentage of bacteria types found in isolated ant species.

Table 2. Sensitivity and resistance profile of bacteria according to antibiotics tested.

Antibiotic	Resistant number	Intermediate number	Sensitive number
Ampicillin	37	13	0
Erythromycin	3	5	42
Gentamicin	0	0	50
Penicillin	27	22	1

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