Journal of Medical Entomology, XX(X), 2020, 1–8
doi: 10.1093/jme/tjaa062
Research

Vector/Pathogen/Host Interaction, Transmission

Ants (Hymenoptera: Formicidae) as Potential Mechanical Vectors of Pathogenic Bacteria in a Public Hospital in the Eastern Amazon, Brazil

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Subject Editor: Michael Rust

Received 11 September 2019; Editorial decision 9 March 2020

Abstract

Studies related to ants found in hospital environments have aroused interest in their role as mechanical vectors of pathogenic microorganisms. The objective of the current research was to determine the species composition and bacterial contamination of ant species found in a public hospital in the eastern Amazonian region. Ants were captured using bait containing honey and sterilized sardines in 15 locations within the Macapá Emergency Hospital, Amapá. Ants were identified morphologically using specific keys. Bacteria were first inoculated in a Brain Heart Infusion broth and then plated on 5% Agar with blood or MacConkey media. Bacterial species were identified through biochemical procedures. In total, 9,687 ants were collected, with 69.8% from the dry season and 30.2% from the rainy season. Nine species of ants were identified belonging to three subfamilies: the *Monomorium pharaonis* (Linnaeus 1758) being the most common, comprising 39.2% of the total specimens. Only one ant species was found in each bait, facilitating microbiological analyses. In total, 92 bacteria isolates were identified comprising 12 species. *Pseudomonas aeruginosa* Schroeter 1872 (Pseudomonadales: Pseudomonadaceae) was pathogenic bacteria, most frequently isolated, comprising 10.9% of the positive samples. The most contaminated ant in the study was *M. pharaonis* with 38.3%. It was the dominant ant species in this hospital environment. Its wide prevalence, forage day and night of this vector in hospital facilitated bacterial contamination. The presence of bacteria on ants may be associated with the dissemination of pathogens which cause hospital infections, making pest control a necessity in these institutions.

Key words: ant, mechanical vectors, *Monomorium pharaonis*, *Pseudomonas aeruginosa*

The study related to ants found in hospital environments has aroused interest since the first reports by Beatson (1972), because of their capacity to transmit intra-hospital infections, being considered a mechanical vector (Pantoja et al. 2009). The presence of these insects in hospitals can be influenced by determining factors such as nest architectural structure, proximity to homes, constant food sources, and intense flow of people (Lise et al. 2006, Lutinski et al. 2015). Hospital infections have been getting the attention of the scientific community due to the high rate of patient mortality. This condition is directly linked with the hospital's sanitary situation and the presence of pathogenic vector microorganisms (Máximo et al. 2014). *Staphylococcus aureus* Rosenbach 1884 (Bacillales: Staphylococcaceae) is considered bacteria which usually is carried by these insects, as well as, there being reports of other microorganisms of the *Serratia* Bizio 1823 (Enterobacteriales: Enterobacteriaceae), *Klebisiella* Trevisan 1885 (Enterobacteriales: Enterobacteriaceae), *Salmonella* Lignieres 1900 (Enterobacteriales: Enterobacteriaceae), *Escherichia* Migula 1895 (Enterobacteriales: Enterobacteriaceae), *Acinetobacter* Brisou and Prévot 1954 (Pseudomonadales: Moraxellacae) and *Enterococcus*

Thiercelin and Jouhaud 1903 (Lactobacillales: Enterococcaceae) (Vieira et al. 2013).

Research done in a hospital at Taubaté- São Paulo indicated that 98.4% of the ants presented microbe growth (Pereira and Ueno 2008). In a research in two hospitals in Divinópolis-Minas Gerais, 10 species of ants were identified and 6 species of pathogenic bacteria were isolated from these ants (dos Santos et al. 2009).

It is important to highlight that the large part of the work regarding this research is concentrated in the south and south-eastern regions of Brazil; in the northern region, there are few studies related to ants as potential mechanical vectors of bacteria in hospitals (de Castro et al. 2015). In the state of Amapá, there are no scientific articles regarding this topic. The objective of this research was to get to determine the bacterial composition and ants species in a public hospital in the eastern Amazonian region.

Materials and Methods

Study Area

The State of Amapá is located in a tropical region, near the Equator, resulting in a warm and humid climate, characterized by the precipitation regimen and subject to great seasonal variations (Tavares 2014). The average temperature ranges from 25°C to 30°C and rainfall rates are higher than 2,500 mm/yr (Aguiar and Naiff 2009). The average relative humidity ranges between 60 and 100% (Tavares 2014). The study was done at the Emergency Hospital in the city of Macapá, Amapá. It is a medium sized hospital which has an emergency unit, adequate for the general treatment of clinical and surgical nature, meeting medium- and high-complexity demands (Maders and Cunha 2015).

Collection and Identification of Ants

The collection of ants was done during the dry season (September, October, and December of 2017) and during rain seasons (January, April, and June of 2018), totalizing a sample effort of 180 collections with 90 baits in each season. Sampling was done with baits in a passive manner, using 50-ml Falcon type tubes, properly identified, and consisting of a rich source of carbohydrates (honey) and another one of protein (sardine). The tubes containing the baits were sterilized using a pan heated to 121°C for 15 min (Pantoja et al. 2009, Máximo et al. 2014). The sampling was done with baits obeyed the following protocol: two during the day, one containing honey and another containing sardine, between 08 AM and 12 PM, and two during the night with the same constituents, during the period between 06 PM and 10 PM at the following locations: Clinic, Medical Clinic, Nursing Station, Medication Room, Semi-Intensive, Pharmacy, Laboratory, Dining Hall, Infirmary 1, 2, 3, and 4, Intensive Care Unit (ICU), Burn Treatment Center (BTC), and Surgical Clinic, being a total of 15 rooms with collection being executed in triplicates. The taxonomic identification of the Formicidae was done in the Arthropod Laboratory of the Federal University of Amapá using specific keys by Fernández (2003) and Baccaro et al (2015).

Using Broth to Cultivate Bacteria Found on Ants

After the collection and identification of the ants, pools of 5–10 individuals were sorted by species, only one ant species was found in each bait and they were deposited in a Brain Heart Infusion (BHI) broth, with the objective of using broth to cultivate bacteria found on ants (Gonçalves et al. 2011, Jacobs and Alves 2014, Máximo et al. 2014). The samples of the BHI broth were shaken lightly to free the highest number of bacteria stuck on the ants body, in order to facilitate the microbial and incubated growth in the green house at 37°C (Oliveira et al. 2017). Microbiological analyzes were performed only with samples of ants collected in the dry season.

Bacterial Isolation

There having been bacterial growth in the BHI broth, aliquots of the broth were removed containing the ant samples and were to seeding in 5% agar blood and MacConkey agar and then incubated for 24 h at 37°C in order to obtain isolated colonies (Tanaka et al. 2007). After the growth, gram staining was performed and seeding of isolated colonies on nonselective agar was performed to obtain pure colonies (Rodovalho et al. 2007, Fontana et al. 2010).

Bacterial Identification

We performed biochemical trials for Gram-Positive Cocci (GPC) (Lima et al. 2013, Jacobs and Alves 2014). For Gram-Negative Bacilli (GNB), first atrial was done in agar trial Triplici Sugar Iron (Rodovalho et al. 2007). The fermenting bacteria were submitted to nine biochemical tests, with some adaptations (Pesquero et al. 2008, Fontana et al. 2010, Jacobs and Alves 2014). A NEWPROV EPM Mili and Citrate kit was used, which contemplate biochemical tests (Brasil 2010). For the identification of the nonfermenters, a commercial name of the brand PROBAC NF III kit was used. The test contained 19 biochemical tests for the identification of the nonglucose fermenting GNBs. The microbiological analysis was executed in the Immunological and Microbiological Laboratory of the Federal University of Amapá.

Data Analysis

Ecological analysis was performed by estimating ant richness, abundance, diversity, equitability, and dominance in different rooms of the Emergency Hospital. Richness refers to the number of species, while abundance relates to the number of individuals. Diversity was estimated through Shannon–Wiener index (H'), which is related to the proportion of individuals of each species. For the equitability calculation, the Pielou index (J') was used, which allows to represent the uniformity of the distribution of the individuals among the collected species. Dominance was assessed using the Berger–Parker index (d), which is the relative frequency of the most abundant species (Southwood 1978, Magurran 2011).

Statistical analysis was performed using the nonparametric Kruskal–Wallis (H) variance test followed by Dunn's complementary test to compare the abundance of ants and bacteria in different hospital rooms. Difference was considered significant when $P < 0.05$. If this test indicates significant difference, a complementary Dunn test will be performed.

The estimation of diversity, equitability, and dominance indices as well as for previously reported statistical analysis was performed using Past software version 1.99 (Hammer et al. 2001).

Results

In the dry season, the average temperature inside the hospital was 32°C with 72% humidity. In the rainy season, the average indoor temperature was 28.2°C with humidity of 87%. In each bait was observed the predominance of only one ant species. In total, 9,687 ants were collected with 69.2% (6.758) from the dry season and 30.8% (2.929) from the rainy season. Three subfamilies were identified: Dolichoderinae, Formicinae, and Myrmicinae, comprising nine species of ants (Table 1). There was no significant difference ($H = 6.958$, $P = 0.6474$) in the abundance of ants between hospital rooms. Of the 15 rooms, only the clinic did not have ants. Infirmary 1, Infirmary 3, Infirmary 4 Laboratory, and Semi-Intensive were the most heaving infested rooms (Table 2).

Table 1. Richness and abundance of species of Formicidae in dry and rainy seasons in Emergency Hospital, Macapá, Amapá, Brazil

Subfamilies/species	Dry		Rainy		Total	%
	Day	Night	Day	Night		
Dolichoderinae	0	85	0	0	85	0.88
Linepithema humile Mayr 1868	559	1,747	426	426	3,158	**32.60**
Tapinoma melanocephalum Fabricius 1793						
Formicinae						
Brachymyrmex sp Mayr 1868	0	190	0	0	190	1.96
Paratrechina longicornis Latreille 1802	259	436	176	328	1,199	**12.38**
Myrmicinae	0	0	20	0	20	0.21
Monomorium floricola Jerdon 1851	2,703	613	60	422	3,798	**39.21**
Monomorium pharaonis Linnaeus 1758						
Solenopsis saevissima Smith Nylander 1846	24	37	601	458	1,120	11.56
Tetramorium bicarinatum Nylander 1846	0	0	0	12	12	0.12
Wasmmania auropunctata Roger 1863	40	65	0	0	105	1.08
Total	3,585	3,173	1,283	1,646	9,687	100

Subfamilies and species of ants identified in the study, demonstrating the Formicidae with greater and lesser frequency. Bold represent higher values of ants found.

Table 2. Distribution of ant species found in rooms in the Emergency Hospital of the city of Macapá-AP

Rooms	*Brachymyrmex* sp.	*Linepithema humile*	*Monomorium pharaonis*	*M. florícola*	*Paratrechina longicornis*	*Solenopsis saevissima*	*Tetramorium bicarinatum*	*Tapinoma melanocephalum*	*W. auropuctata*
Surgical Clinic	0	0	744	0	0	0	0	70	0
Medical Clinic	0	0	0	0	180	0	0	0	0
Clinic	0	0	0	0	0	0	0	0	0
BTC	0	0	0	0	13	0	0	138	0
Infirmary 1	0	85	935	0	263	0	12	253	0
Infirmary 2	0	0	250	0	0	0	0	104	0
Infirmary 3	0	0	1,110	0	91	0	0	7	0
Infirmary 4	0	0	0	0	545	0	0	665	0
Pharmacy	0	0	50	0	0	24	0	0	0
Laboratory	0	0	210	0	0	0	0	1,206	105
Nursing Post	0	0	301	0	0	80	0	534	0
Dining hall	190	0	0	0	0	0	0	0	0
Semi intensive	0	0	191	0	0	791	0	31	0
Medication Room	0	0	7	20	107	225	0	140	0
ICU	0	0	0	0	0	0	0	10	0
Total	190	85	3,798	20	1,199	1,120	12	3,158	105

In the Medication Room, a greater index of diversity (H' = 1,235) was estimated. In the Medical Clinic, Dining hall, and ICU, greater dominance was verified (d = 1). In regards to equitability among rooms, the greater index (j = 0.9929) was observed in Infirmary 4 (Table 3).

Microbiological Analysis

In dry seasons, of the 90 baits used, 53 (58.9%) presented ants, 53 pools were done for microbiological trial in BHI broth. In total, 92 microorganisms were identified divided into 12 species distributed in 15 rooms (Table 4). There was significant difference (H = 16.94 and P = 0.03921) among the rooms regarding isolated bacterial, being 35 (38.04%) Positive-Gram Bacillis (PGB), 31 (33.7%) GNB, and 26 (28.26%) GPC were identified. Completion by Dunn's test indicating the rooms that present significant difference in relation to the occurrence of bacteria (Table 5). Infirmary 1 was the rooms considered the most contaminated, with 10.9%, and the most frequent pathogenic bacteria in the study were *Pseudomonas aeruginosa*.

The number of isolated bacteria varied in relation to the different species of ants collected with 36 (39.13%) in *Monomorium pharaonis*, 21 (22.83%) in *Tapinoma melanocephalum* Fabricius 1793, 20 (21.74%) in *Paratrechina longicornis* Latreille 1802, 10 (10.87%) in *Solenopsis saevissima* Smith 1855, 3 (3.26%) in *Wasmmania auropunctata* Roger 1863, and 1 (1.09%) for both *Linepithema humile* Mayr 1868 and *Brachymyrmex* sp. (Fig. 1).

Discussion

Interaction of Ants With the Hospital Environment

In the current study, greater richness and an abundance in Formicidae was observed in comparison to similar works in another hospitals (Silvestre et al. 2014, Lutinski et al. 2015, Oliveira et al. 2017). The elevated abundance and richness obtained in this research probably was related to important factors such as the internal temperature

Table 3. Ecological indices of ants found in Emergency Hospital sectors

Ecological indices	SC	MC	Cli	BTC	Inf 1	Inf 2	Inf 3	Inf 4	Phar	Lab	NP	DH	SI	MR	ICU
Shannon–Wiener H'	0.2932	0	0	0.2934	**1.062**	0.6055	0.3024	0.6882	0.9463	0.6419	0.8776	0	0.6144	**1.235**	0
Equitabilyti *J*	0.423			0.4233	0.7658	0.8736	0.2752	**0.9929**	0.8613	0.5843	0.7988		0.5593	0.7672	
Berger–Parker *d*	0.914	**1**	0	0.9139	0.6087	0.7062	0.9189	0.5496	0.5814	0.7929	0.601	**1**	0.7808	0.4509	**1**

SC, Surgical Clinic; MC, Medical Clinic; Cli, Clinic; BTC, Burn Treatment Center; Inf 1, Infirmary 1; Inf 2, Infirmary 2; Inf 3, Infirmary 3; Inf 4, Infirmary 4; Phar, Pharmacy; Lab, Laboratory; NP, Nursing Post; DH, Dinning hall; SI, Semi-Intensive; MR, Medication Room; ICU, Intensive Care Unit.

Bold represent the values of the highest indexes.

Table 4. Occurrence of bacteria associated to the ants by sectors of the Emergency Hospital

Bacteria/rooms	SC	MC	Cli	BTC	Inf 1	Inf 2	Inf 3	Inf 4	Phar	Lab	NP	DH	SI	MR	ICU	Total	%
Bacillus sp. Cohn 1872	2	2	0	2	3	2	3	3	3	2	5	1	3	4	0	35	38.04
Escherichia coli Migula 1895	1	2	0	1	1	0	0	1	0	0	0	0	1	0	0	7	7.61
Enterobacter sp. Hormaeche and Edwards 1960	1	1	0	1	1	0	1	1	0	1	0	0	1	0	0	8	8.70
Enterococcus sp. Thiercelin and Jouhaud, 1903	1	0	0	0	1	1	0	0	0	1	1	0	0	0	0	5	5.43
Klebisiella sp. Trevisan 1885	1	1	0	0	1	0	1	0	0	1	0	0	1	0	0	6	6.52
Staphylococcus aureus Rosenbach 1884	0	0	0	0	1	0	1	0	0	0	1	0	0	1	0	4	4.35
Staphylococcus epidermidis Evans 1916	0	1	0	2	0	0	0	1	0	1	0	0	0	0	0	5	5.43
Staphylococcus capitis Kloos and Schleifer 1975	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	2	2.17
Staphylococcus haemolyticus Schleifer and Kloos 1975	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	5	5.43
Staphylococcus cohii Schleifer and Kloos 1975	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1.09
Staphylococcus saprophyticus Fairbrother 1940	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	4	4.35
Pseudomonas aeruginosa Schroeter 1872	1	0	0	1	1	1	0	0	1	1	0	1	2	1	0	10	10.87
Total	8	9	0	7	10	5	6	8	5	8	8	2	9	7	0	92	100

SC, Surgical Clinic; MC, Medical Clinic; Cli, Clinic; BTC, Burn Treatment Center; Inf 1, Infirmary 1; Inf 2, Infirmary 2; Inf 3, Infirmary 3; Inf 4, Infirmary 4; Phar, Pharmacy; Lab, Laboratory; NP, Nursing Post; DH, Dinning hall; SI, Semi-Intensive; MR, Medication Room; ICU, Intensive Care Unit.

Table 5. Dunn's test of variance analysis of the occurrence of isolated bacteria from ants in the Emergency Hospital rooms, Macapá, Amapá, Brazil

	SC	MC	Cli	BTC	Inf 1	inf 2	Inf 3	Inf 4	Phar	Lab	NP	DH	SI	MR	ICU
SC		0.8914	**0.01112**	0.5331	0.6822	0.2544	0.2669	0.7261	0.1362	1	0.2748	**0.04174**	0.8307	0.2728	**0.01112**
MC	0.8914		**0.007464**	0.4473	0.7849	0.2019	0.2125	0.6264	0.1038	0.8914	0.2193	**0.02981**	0.7261	0.2176	**0.007464**
Cli	**0.01112**	**0.007464**		0.05542	**0.003194**	0.1618	0.1531	**0.02863**	0.2943	**0.01112**	0.1479	0.6151	**0.02007**	0.1492	1
BTC	0.5331	0.4473	0.05542		0.3017	0.6056	0.6264	0.7849	0.3861	0.5331	0.6393	0.1577	0.6822	0.6361	0.05542
Inf 1	0.6822	0.7849	**0.003194**	0.3017		0.1213	0.1286	0.4473	0.05748	0.6822	0.1332	**0.01446**	0.5331	0.1321	**0.003194**
inf 2	0.2544	0.2019	0.1618	0.6056	0.1213		0.9764	0.4299	0.7261	0.2544	0.9619	0.3701	0.3545	0.9655	0.1618
Inf 3	0.2669	0.2125	0.1531	0.6264	0.1286	0.9764		0.4473	0.704	0.2669	0.9855	0.3545	0.3701	0.9891	0.1531
inf 4	0.7261	0.6264	**0.02863**	0.7849	0.4473	0.4299	0.4473		0.2544	0.7261	0.4583	0.09184	0.8914	0.4555	**0.02863**
Phar	0.1362	0.1038	0.2943	0.3861	0.05748	0.7261	0.704	0.2544		0.1362	0.6905	0.5851	0.2019	0.6939	0.2943
Lab	1	0.8914	**0.01112**	0.5331	0.6822	0.2544	0.2669	0.7261	0.1362		0.2748	**0.04174**	0.8307	0.2728	**0.01112**
NP	0.2748	0.2193	0.1479	0.6393	0.1332	0.9619	0.9855	0.4583	0.6905	0.2748		0.3451	0.3799	0.9964	0.1479
DH	**0.04174**	**0.02981**	0.6151	0.1577	**0.01446**	0.3701	0.3545	0.09184	0.5851	**0.04174**	0.3451		0.06841	0.3474	0.6151
SI	0.8307	0.7261	**0.02007**	0.6822	0.5331	0.3545	0.3701	0.8914	0.2019	0.8307	0.3799	0.06841		0.3774	**0.02007**
MR	0.2728	0.2176	0.1492	0.6361	0.1321	0.9655	0.9891	0.4555	0.6939	0.2728	0.9964	0.3474	0.3774		0.1492
ICU	**0.01112**	**0.007464**	1	0.05542	**0.003194**	0.1618	0.1531	**0.02863**	0.2943	**0.01112**	0.1479	0.6151	**0.02007**	0.1492	

SC, Surgical Clinic; MC, Medical Clinic; Cli, Clinic; BTC, Burn Treatment Center; Inf 1, Infirmary 1; Inf 2, Infirmary 2; Inf 3, Infirmary 3; Inf 4, Infirmary 4; Phar, Pharmacy; Lab, Laboratory; NP, Nursing Post; DH, Dinning hall; SI, Semi-Intensive; MR, Medication Room; ICU, Intensive Care Unit.

Bold represent *P* values greater than 0.05.

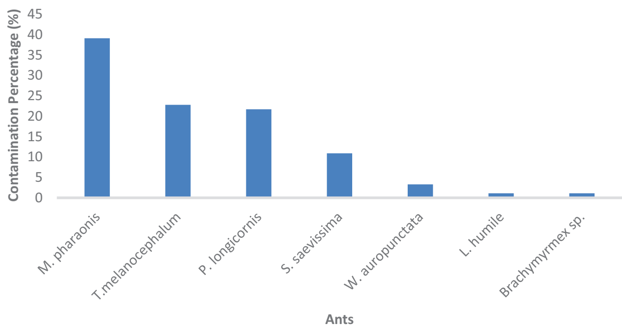

Fig. 1. Percentage of ants contaminated by bacteria in Emergency Hospital, Macapá, Amapá, Brazil.

of the hospital may be higher than the external, abundant presence of food, conditions regarding the precarious architectural structure of the hospital, and the lack of air conditioning stimulating the ants to remain there (Lima et al. 2013). Even though ants fauna is considered as varied, exotic Formicidae were predominant (Gazeta et al. 2008). The most exotic species found in Brazilian hospitals are *P. longicornis*, *T. melanocephalum*, *Monomorium floricola* (Jerdon 1851), *M. pharaonis*, and *Pheidole megacephala* Fabricius 1973 (Bragança and Lima 2010).

At the room with high abundance, it was observed that Infirmary 1 has demonstrated the best conditions for the permanence of the ants: as places to build nest, locomotion free of predators, and proximity to other rooms, making the flow of Formicidae easy from one room to another.

Monomorium pharaonis is considered an indoor species, but momentarily, it will leave to forage in the external areas of the building. They build nests in cavities outside and inside the buildings. Their frequency in hospital rooms can be attributed to their habits and ecological characteristics. It is a dominant species in hospital environments (Lutinski et al. 2015). This ant has been found in greater numbers and with various bacterial isolations (Tanaka et al. 2007, Alharbi et al. 2019).

Tapinoma melanocephalum is a *'tramp ant'* found all over the world, especially in tropical and subtropical climates. There are records related to high degree of infestation in hospitals (Guerrero 2018) and this is the only species in several rooms of hospitals in Porto Velho, RO (Vieira et al. 2013), Uberaba, MG (Teixeira et al. 2009), and most abundance in two public hospitals at Fortaleza, CE (Pantoja et al. 2009).

Paratrechina longicornis has unstructured nests and with the smallest sign of disturbance can have their whole colonies fragmented and consequently will migrate to other areas spreading in to various rooms (Bragança and Lima 2010). This species *P. longicornis* was predominant in another work (Shahi et al. 2017). The ants of the *Solenopsis* gender can cause accidents through their jaws and strings, causing injuries (Pitts et al. 2005, Delabie 2009, Guarda et al. 2018).

The ant fauna recorded in Brazil presents a potential to act as a mechanical vector for the spreading of pathogenic microorganisms in hospitals, being greater if compared with European countries. The invasive species such as *M. pharaonis*, *T. melanocephalum*, and *P. longicornis* are considered the most dominant in these environments (Mello Garcia and Lise 2013). The presence of these three species in this study reinforces its adaptation in a nosocomial environment. Probably, what corroborates for that is the climate of the Amazonic region, because these insects have preference for a tropical and subtropical climate, which eases the foraging and nidification in hospital as well as residential environments (Guerrero 2018).

The ICU is an environment for the hospitalization of patients in critical conditions, in need of specialized assistance (Ferreira et al. 2016). The incidence of the *M. pharaonis* species, competent microorganism vector, presents a severe issue. A different result was obtained in a hospital denominated as 'H5' in Porto Alegre, RS, which had 40% of this incidence registered in the ICU, predominantly being the *T. melanocephalum* (Fonseca et al. 2010). Semi-Intensive is a room destined to patients which are in critical condition that are waiting for a vacancy to be transferred to in the ICU (Silva et al. 2012). In a research done in Bahia, *T. malenocephalum* was dominating in this room. Nests of these ants found in baseboards and holes in the wall (Oliveira et al. 2017).

Infirmary 1 was one of the rooms with greatest abundance of ants. Similar results were found in other studies (Fonseca et al. 2010, Silva et al. 2012). Dining hall, floor cleaning was performed periodically, which drastically reduced the number of ants in this rooms, and only one species was found. The invasive ants (*M. pharaonis*, *T. melanocephalum*, and *P. longicornis)* present some characteristics, such as unicolonialism (Bandoy and Tiu 2017), polygyny, and colony fragmentation (Bragança and Lima 2010) that allows their dispersion and adaptation in different anthropic environments, being these characteristics considered key attributes for the ecological success of the invading ants.

The Relation of Ants With the Pathogenic Bacteria Identified in the Study

The occurrence of pathogenic bacteria was relevant, as they were reported in almost all rooms, except the clinic and the ICU. The room with the greatest index of contamination was Infirmary 1, with 10 isolated bacteria. The more there are people roaming the rooms of the hospital, greater is the possibility of finding ants circulating in search of food, especially close to the trash bins within the Infirmary (Bicho et al. 2007).

The bacterial diversity carried by these ants is a potential source for the spread of these pathogenic bacteria in hospital rooms. Infirmary that takes patients in and that performs periodical change of bandages presents a higher possibility of acquiring hospital infections, as well as to disseminate pathogens in these locations to the patients and consequently to the ants found there.

The study found at the hospital environment 16 *Staphylococcus* strains, being three (18.75%) *S. aureus* found in the room of food preparation and Infirmary and 13 (81.25%) belonged to the group Coagulase-Negative *Staphylococcus* (CNS) of which include *Staphylococcus epidermidis* Evans 1916 (Bacillales: Staphylococcaceae) *and Staphylococcus saprophyticus* Fairbrother 1940 (Bacillales: Staphylococcaceae) found in all the rooms of hospital. In addition, enterobacteria have been found, being *Escherichia coli* Migula 1895 and *Klebisiella* sp. were isolated from the ICU, Dining Hall, and Semi-Intensive (Oliveira et al. 2017). The CNS are colonizers of skin and mucosal surfaces; they frequently infect catheters inserted in critically ill patients. Are opportunistic pathogen (Shi et al. 2019). In another study, it was possible to isolate 18 microorganisms from seven ant species (Menezes et al. 2015). We have identified more bacteria species carried for ants that other similar studies. The significant difference in the occurrence of bacterial species in different rooms of the hospital ($H = 16.94$ and $P = 0.03921$) may be related to aspects associated with the ability of ants to disperse in these environments.

Pseudomonas aeruginosa is considered ubiquitous and one of the main etiological agents that cause hospital infections. This microorganism has been responsible for the majority of hospital pneumonia, venous, and bladder catheterization infections (Lima et al. 2013, Máximo et al. 2014, Soares et al. 2016, Alves et al. 2017).

Enterobacter sp. are encapsulated pathogens which ferment lactose. They are frequently associated to various cases of hospital infections such as urinary tract infection, pneumonia, and colonization of wounds. They have the capacity of contaminating hospital devices for invasive procedures (Correa et al. 2018). This pathogen was one of the most associated with hospital infections and showed a 100% resistance to the antibiotics tested (Lise et al. 2006). In another study, isolated ant bacteria, 10% belonged to this strain (Amarsy et al. 2019).

Escherichia coli is a bacterium which is constantly cause urinary tract infections (Hespanhol et al. 2017). In a similar study, the result was elevated, because 18% belonged to this species were isolated from ants (Simothy et al. 2018).

Klebisiella pneumoniae Schroeter 1886 is a pathogen which established itself in the nosocomial environment effectively. It is a bacterium highly dangerous to newborns and individuals undergoing intensive treatment, especially the strain producing carbapenemases (Miranda et al. 2018). The presence of this bacteria associated to ants was reported in other studies (Teixeira et al. 2009, Lima et al. 2013, Máximo et al. 2014).

Staphylococcus aureus, this pathogen can survive in inanimate surfaces during long periods, which increases the possibility of infection in certain individuals. It is also considered the most frequent pathogen in the hospital environment with elevated virulence (Lestari et al. 2019). Research done in São Luis - Maranhão found that 12.4% of them isolated with the bacteria *S. aureus* (Lima et al. 2013), demonstrating a percentage three times what was found in our research.

The three ant species that was most frequently found in this study were also the ones that were the most contaminated by bacteria. It is important to highlight that the 12 different isolated etiological agents were found associated to *M. pharaonis* with this ant considered as the most contaminated.

Studies in Indonesia found four strains of microorganisms associated only to *M. pharaonis* (Lestari et al. 2019) and elevated levels of contamination were associated with *T. melanocephalum* with 60 species of bacteria (Teixeira et al. 2009).

The ants forage the hospital and return to the colony contaminating each other, which might be one of the causes for the high amount of pathogens associated to these insects. It can be said that, the greater the abundance of ants in the hospital, the greater the probability microorganisms contamination in these Formicidae (Fontana et al. 2010).

In the predominance of only one ant species among all others that compete in the same ecologic niche, the dissemination of pathogenic bacteria may take place in a more accentuated fashion. In this social structure, the free movement of the worker ants throughout the different rooms of the hospital under study is enabled. Therefore, these Formicidae may acquire pathogens at highly contaminated environments, disseminating them to other areas (Garcia et al. 2014).

The existence of these pathogens on Formicidae can be related to the presence of these bacteria in the clinical isolation of the hospitalized patients (Teixeira et al. 2009). This condition may associate the ant as a potential mechanical vector of pathogenic bacteria in hospital settings (Rodovalho et al. 2007). The implementation of a pest control strategy continuously within this institution is essential to reduce the amount of Formicidae (dos Santos et al. 2009). Further studies are needed to better understand the relationship of bacteria with ants, as well as a better understanding of ant biology and habits in these unnatural environments. These results serve as an alert, because the preoccupation of only the hospital infection

is being observed, which have as transmitters, health professionals, visitors, and even patients.

Acknowledgments

We thank the colleagues that contributed to this research and the Federal University of Amapá.

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