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Author(s): L.D.M. Pantoja, R. E. Moreira Filho, E.H.S. Brito, T. B. Aragão, R.S.N. Brilhante, R. A. Cordeiro, M.F.G. Rocha, A. J. Monteiro, Y. P. Quinet, and J.J.C. Sidrim

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Ants (Hymenoptera: Formicidae) as Carriers of Fungi in Hospital Environments: An Emphasis on the Genera *Tapinoma* and *Pheidole*

L.D.M. PANTOJA,^{1,2,3} R. E. MOREIRA FILHO,^{1,2} E.H.S. BRITO,^{1,4} T. B. ARAGÃO,^{5,6}
R.S.N. BRILHANTE,^{1,2,7} R. A. CORDEIRO,^{1,2,5} M.F.G. ROCHA,^{1,2,4} A. J. MONTEIRO,⁸
Y. P. QUINET,^{5,6} AND J.J.C. SIDRIM^{1,2}

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ABSTRACT The aim of this study was to evaluate the presence of filamentous fungi and yeasts on the external surface of ants at hospitals. From March 2007 to February 2008, 2,899 ants were evaluated in two public hospitals in the city of Fortaleza, Ceará, in northeastern Brazil. The ants were attracted by nontoxic baits, distributed within critical and semicritical hospital areas. The fungi were identified through macro- and micromorphological analysis, biochemical profile, and growth in chromogenic medium. From this study, 5 genera and 13 species of ants were identified, from critical (8% of the collected ants) and semicritical (92%) areas, during the daytime (48%) and nighttime (52%) periods. In the mycological analysis, 75% of the ants were fungi carriers, with the species *Tapinoma melanocephalum* and species from the genus *Pheidole* having the most potential as carriers of airborne fungi (75 and 18%, respectively) and yeasts (6 and 1%, respectively). In summary, ants act as carriers of airborne fungi and yeasts, including some pathogenic species.

KEY WORDS ants, *Tapinoma*, *Pheidole*, fungi, hospital environment

Arthropods correspond to 75% of the earth's animals, and 89% of them are insects (Brusca and Brusca 2003). They are the group with the greatest number of species distributed in temperate and particularly tropical countries (Chacón de Ulloa 2003).

Because of the close association of insects with humans, their role as potential mechanical carriers of pathogenic microorganisms has been studied. Some authors have reported adult insects and nymphs carrying viruses, bacteria, fungi, and protozoa, as well as serving as intermediate hosts of helminthes (Robinson 1996, Mariconi 1999), thus posing a potential danger to public health.

This risk can be associated with different insects, with emphasis on the following orders: Diptera (flies and mosquitoes) (Fotadar 2001, Graczyk et al. 2003), Blattodea (cockroaches) (Imamura et al. 2003, Lemos et al. 2006), and Hymenoptera (bees, wasps, and ants). Because of their dominant behavior, ants can cause

serious problems to humans, and some are considered urban pests. Great efforts have been made to control ants, but most of the results have only temporary effects (Bueno and Campos-Farinha 1999).

Since the first reports of the African species *Monomorium pharaonis* in European hospitals (Beatson 1972), the possible effects on human health of ants in hospitals roused interest because of the great adaptability and the free contact of these insects with hospital material, wastes, and patients themselves, and their consequent capacity to transmit pathogenic microorganisms. Therefore, ants can act as an important source of hospital bacterial infection (Zarzuela et al. 2002, Chacón de Ulloa 2003).

Ant monitoring in hospital environments is still rare and unevenly distributed (Chacón de Ulloa 2003). Hospitals in tropical countries have classified environments according to the risk of hospital infection into critical and semicritical areas. Critical areas offer potential risk of infection, characterized by closed environments with air conditioning or other climate control, although semicritical areas are occupied by patients who do not need intensive care or isolation and are generally open environments without climate control (Brasil 1985).

Some studies have shown that ants can carry bacteria such as *Acinetobacter* spp., *Corynebacterium* spp., *Enterobacter* spp., *Enterococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Serratia* spp., and *Staphylococcus* spp. (Lise et al. 2006, Rodvalho et al. 2007). Concerning fungi, there are reports of the presence of

¹ Specialized Medical Mycology Center, Federal University of Ceará, Ceará, Brazil.

² Postgraduate Program in Medical Microbiology, Federal University of Ceará, Ceará, Brazil.

³ Corresponding author: Rua Otávio Costa 28, Edson Queiroz, CEP 60812-410, Fortaleza CE, Brazil (e-mail: lydiapantoja@bol.com.br).

⁴ Postgraduate Program in Veterinary Science, State University of Ceará, Ceará, Brazil.

⁵ Department of Biological Science, State University of Ceará, Ceará, Brazil.

⁶ Laboratory of Entomology, State University of Ceará, Ceará, Brazil.

⁷ Corresponding author, e-mail: brilhante@ufc.br.

⁸ Department of Statistics and Applied Mathematics, Federal University of Ceará, Ceará, Brazil.

Table 1. Number of ants captured per species in the various sectors of two hospitals in the city of Fortaleza, Ceará, Brazil, from Mar. 2007 to Feb. 2008

Ant species	Hospital environment									
	Critical areas					Semicritical areas				
	Hospital A IF (n)		Hospital B IF (n)			Hospital A IF (n)			Hospital B IF (n)	
	ICU	SC	ICU	ICUneoI ^a	ICUneoII ^a	PW	HW	TW	SW	GW
<i>Tapinoma melanocephalum</i>	13	13	—	78	91	260	260	247	299	312
<i>Paratrechina longicornis</i>	—	—	—	26	—	52	52	78	130	130
<i>Pheidole impressa</i>	—	—	—	—	—	65	13	—	—	—
<i>Pheidole radoszkowskii</i>	—	—	—	—	—	26	—	—	—	—
<i>Pheidole</i> sp. <i>diligens</i> group	—	—	—	—	—	—	—	—	—	78
<i>Pheidole</i> sp. <i>fallax</i> group	—	—	—	—	—	169	—	—	—	—
<i>Solenopsis globularia</i>	—	—	—	—	—	52	—	13	26	39
<i>Solenopsis saevissima</i>	—	—	—	—	—	13	13	—	—	—
<i>Solenopsis</i> sp.	—	—	—	—	—	—	—	—	—	13
<i>Camponotus arboreus</i>	—	—	—	—	—	—	104	—	—	—
<i>Camponotus melanoticus</i>	—	—	—	—	—	—	13	13	—	—
<i>Camponotus renggeri</i>	—	—	—	—	—	—	—	—	—	13
<i>Camponotus vittatus</i>	—	—	—	—	—	—	91	104	—	—
Total	13	13	—	104	91	637	546	455	455	585

^a Two neonatal ICUs at the same hospital, but in different buildings.

—, absence; n, no. of ants captured; ICU, adult intensive care unit; SC, surgery center; ICUneoI, neonatal intensive care unit I; ICUneoII, neonatal intensive care unit II; PW, pediatrics ward; HW, hematology ward; TW, transplant ward; SW, surgery ward; GW, gynecology ward.

airborne fungi such as *Aspergillus* spp., *Penicillium* spp., and *Cladosporium* spp. (Barros et al. 2006), as well as yeasts, especially from the genus *Candida* spp. (Barros et al. 2006).

The increase of opportunistic fungal infections has led to a search for the sources of contamination inside hospital environment (Perdelli et al. 2006). However, few studies are available on the real involvement of ants as fungus carriers. The aim of this study was to evaluate the presence of filamentous fungi and yeasts on the external surface of ants at public hospitals in northeastern Brazil.

Materials and Methods

The study was conducted in two public hospitals (hospital A and hospital B), which act in tertiary care, education, and research and are both located in the city of Fortaleza, Ceará. In each hospital, five sectors were analyzed, grouped according to the risk of hospital infection (Brasil 1985): critical areas (intensive care unit [ICU] and surgical ward) and semicritical areas (infirmaries). This study was submitted to analysis of the research ethics committees of the two institutions and obtained consent in February 2007.

From March 2007 to February 2008, ants were collected on a monthly basis, just after cleaning activities in the daytime and just after the changeover to the nighttime shift. A total of 2,899 ants were analyzed, collected during the day (1,391) and night (1,508).

The ants were attracted by nontoxic and sterilized baits, consisting of honey and commercial cat food (Zarzuela et al. 2002), with the addition of sterile cotton. Fifteen baited traps were set out in each hospital sector. Two samples were collected from each positive bait (with ants): one with 3 ants for entomological identification (total of 669 ants) and another

with 10 ants for mycological analysis (total of 2,230 ants) (Moreira et al. 2005).

The ants were mounted with entomological pins and examined under a stereoscopic microscope for identification using identification keys (Bolton 1994).

To isolate the filamentous fungi and yeasts, in each sample with 10 ants, 1.0 ml of saline solution was added to each sample of 10 ants. Aliquots were distributed on the surface of the following culture media: Sabouraud agar medium (Sanofi, Paris, France) supplemented with vancomycin (0.1 g/liter) and polymyxin B (0.0075 g/liter), caffeic acid agar medium, and Niger agar medium. These last two media were used to assist in the presumptive identification of the genus *Cryptococcus* (Vidotto et al. 2004, Pedroso et al. 2007). The culture media were incubated for 15 d at 26–28°C and examined on a daily basis.

Filamentous fungi were identified by macroscopic and microscopic examination of the characteristics of fungal colonies with the aid of identification keys (Hoog et al. 2000). For identification of yeast, the following tests were conducted: germinative tube, microculture in cornmeal agar with Tween 80 (Difco, Detroit, MI), growth in CHROMagar (Candida, Paris, France), auxanogram, zimogram subcultures, and nitrogen assimilation (Gautret et al. 2000, Hoog et al. 2000).

Results

Five genera and 13 species of ants were identified. Species from the genera *Tapinoma* ($n = 1,573$ ants) and *Paratrechina* ($n = 468$) were the most abundant, followed by *Pheidole* ($n = 351$), *Camponotus* ($n = 338$), and *Solenopsis* ($n = 169$).

These genera were distributed in critical (8% of collected ants) and semicritical areas (92%) (Table 1). The species *T. melanocephalum* and *P. longicornis* were

Table 2. Association between no. of fungi isolated and ants genera sampled monthly in hospital A and hospital B from Mar. 2007 to Feb. 2008

Fungal species	Ant genera and hospital									
	<i>Tapinoma</i>		<i>Paratrechina</i>		<i>Pheidole</i>		<i>Solenopsis</i>		<i>Camponotus</i>	
	A	B	A	B	A	B	A	B	A	B
Airborne fungi										
<i>Absidia</i> sp.	2	—	—	—	1	—	—	—	—	—
<i>Acremonium</i> sp.	1	1	—	—	2	—	1	—	—	—
<i>Acremonium hyalinulum</i>	3	1	1	—	—	—	—	—	—	—
<i>Aspergillus flavus</i>	22	16	1	4	8	4	2	1	1	—
<i>Aspergillus niger</i>	—	7	—	1	—	—	—	—	1	—
<i>Aspergillus ochraceus</i>	21	26	2	5	5	3	3	1	—	—
<i>Aspergillus oryzae</i>	3	5	—	1	1	1	1	1	—	—
<i>Aspergillus sydowii</i>	2	—	1	—	—	—	—	—	—	—
<i>Aspergillus versicolor</i>	2	—	—	—	—	—	—	—	—	—
<i>Chrysosporium inops</i>	2	1	1	1	—	—	—	1	—	—
<i>Cladosporium sphaerospermum</i>	8	5	2	—	6	1	—	—	1	—
<i>Cokeromyces</i> sp.	4	—	—	—	—	—	—	—	—	—
<i>Cunninghamella bertholletiae</i>	8	8	1	—	1	1	1	1	—	—
<i>Cyphellophora</i> sp.	2	—	—	—	—	—	—	—	—	—
<i>Fusarium</i> sp.	—	—	1	—	1	—	1	—	—	—
<i>Fusarium proliferatum</i>	2	—	—	—	—	—	—	—	—	—
<i>Fusarium solani</i>	—	1	—	—	—	—	—	—	—	—
<i>Mortierella polycephala</i>	—	—	—	1	2	—	—	—	—	—
<i>Mucor</i> sp.	3	1	—	—	—	—	—	—	—	—
<i>Mycocentrospora acerina</i>	1	—	—	—	1	—	—	—	—	—
<i>Ochroconis gallopava</i>	2	—	—	—	—	—	—	—	—	—
<i>Paecilomyces marquandii</i>	1	1	—	—	—	—	—	—	—	—
<i>Paecilomyces variotii</i>	2	1	—	—	1	—	—	—	—	—
<i>Penicillium</i> sp.	20	25	4	3	7	—	1	1	—	—
<i>Rhinocladiella aquaspersa</i>	—	2	—	—	1	—	—	—	—	—
<i>Scopulariopsis koningii</i>	2	—	—	—	—	—	—	—	—	—
<i>Scytalidium</i> sp.	—	—	—	—	1	—	—	—	—	—
<i>Tritirachium oryzae</i>	1	—	—	—	—	—	—	—	—	—
Yeasts										
<i>Candida albicans</i>	2	1	—	—	—	—	—	—	—	—
<i>Candida glabrata</i>	1	—	—	—	—	—	—	—	—	—
<i>Candida guilliermondii</i>	1	—	—	—	1	—	—	—	—	—
<i>Candida parapsilosis</i>	3	4	—	—	—	1	—	—	—	—
<i>Rhodotorula</i> sp.	2	—	—	—	—	—	—	—	—	—
<i>Rhodotorula mucilaginosa</i>	—	—	—	—	1	—	—	—	—	—
<i>Saccharomyces</i> sp.	—	3	—	—	—	—	—	—	—	—

—, absence.

the most frequent in semicritical areas (68 and 22%, respectively) and critical areas (10 and 2%, respectively). Concerning collection period, 48% of the ants were collected during the day and 52% at night.

The mycological analysis showed that 1,660 (75%) ants were fungus carriers. *T. melanocephalum* and species from the genus *Pheidole* had the highest potential as carriers of airborne fungi (75 and 18%, respectively) and yeasts (6 and 1%, respectively). Of the fungi identified, airborne fungi (94%) were the most frequent on the external surface of the ants. Only 6% of the ants carried yeasts (Table 2).

Regarding the distribution, in each hospital environment, of the fungi isolated from ants, a higher frequency of airborne fungi and yeasts in semicritical areas was observed (83 and 6%, respectively) than in critical areas (only 11% of airborne fungi; Table 3).

Finally, analysis of the evolution of the infestation index (relative frequency of positive baits) in both hospitals as a function of monthly rainfall showed no significant statistical relationship (χ^2 test, $P > 0.05$).

Discussion

In hospitals in the southeastern and southern regions of Brazil, the presence of ants of the genera *Tapinoma*, *Paratrechina*, *Pheidole*, *Solenopsis*, and *Camponotus* has been reported (Zarzuela et al. 2002, Lise et al. 2006). These genera are known as typically associated with urban environments (Passera and Aron 2005). The taxonomic structure of fauna shown in these studies is similar to the one found in both hospitals in this research.

Of the 13 species identified, the two most frequent (*T. melanocephalum* and *P. longicornis*) are considered the two most often occurring in urban/hospital environments in Brazil and other South American countries and are responsible for the highest number of complaints and concern in these environments (Chacón de Ulloa 2003, Moreira et al. 2005). They possess biological characteristics that favor their establishment and propagation in urban environments, such as highly polygynic colonies, small size of workers and queens, generalist diet, great flexibility concerning nesting places, reproduction by

Table 3. Distribution of fungi isolated from the external surface of ants collected from several sectors of two hospitals in the city of Fortaleza, Ceará, Brazil, from Mar. 2007 to Feb. 2008

Fungal species	Hospital environmental									
	Critical areas					Semicritical areas				
	Hospital A		Hospital B			Hospital A			Hospital B	
	ICU	SC	ICU	ICUneoI ^a	ICUneoII ^a	PW	HW	TW	SW	GW
Airborne fungi										
<i>Absidia</i> sp.	—	—	—	—	—	1	2	—	—	—
<i>Acremonium</i> sp.	—	—	—	—	1	2	1	1	—	—
<i>Acremonium hyalinulum</i>	—	—	—	—	—	1	1	2	—	1
<i>Aspergillus flavus</i>	1	—	—	4	1	16	12	4	7	14
<i>Aspergillus niger</i>	—	—	—	1	1	—	1	—	3	3
<i>Aspergillus ochraceus</i>	1	—	—	5	3	10	8	11	11	17
<i>Aspergillus oryzae</i>	—	—	—	—	2	2	2	1	2	4
<i>Aspergillus sydowii</i>	—	—	—	—	—	—	1	2	—	—
<i>Aspergillus versicolor</i>	—	—	—	—	—	—	—	2	—	—
<i>Chrysosporium inops</i>	—	—	—	—	—	1	—	2	2	1
<i>Cladosporium</i>	—	—	—	1	1	11	2	4	1	3
<i>sphaerospermum</i>										
<i>Cokeromyces</i> sp.	—	—	—	—	2	—	—	2	—	—
<i>Cunninghamella</i>	—	—	—	1	1	1	4	5	6	3
<i>bertholletiae</i>										
<i>Cyphellophora</i> sp.	—	—	—	—	—	—	—	2	—	—
<i>Fusarium</i> sp.	—	—	—	—	—	3	—	—	—	—
<i>Fusarium proliferatum</i>	—	—	—	—	—	—	1	1	—	—
<i>Fusarium solani</i>	—	—	—	—	—	—	—	—	—	1
<i>Mortierella polycephala</i>	—	—	—	—	—	2	—	—	1	—
<i>Mucor</i> sp.	—	—	—	—	—	1	1	1	1	—
<i>Mycocentrospora acerina</i>	—	—	—	—	—	1	1	—	—	—
<i>Ochroconis gallopava</i>	—	—	—	—	—	2	—	—	—	—
<i>Paecilomyces marquandii</i>	—	—	—	—	—	1	—	—	1	—
<i>Paecilomyces variotii</i>	—	—	—	—	—	1	—	2	1	—
<i>Penicillium</i> sp.	—	1	—	3	5	14	9	8	12	9
<i>Rhinocladiella aquaspersa</i>	—	—	—	1	—	1	—	—	1	—
<i>Scopulariopsis koningii</i>	—	—	—	—	—	—	—	2	—	—
<i>Scytalidium</i> sp.	—	—	—	—	—	1	—	—	—	—
<i>Tritirachium oryzae</i>	—	—	—	—	—	—	—	1	—	—
Yeast										
<i>Candida albicans</i>	—	—	—	—	—	2	—	—	1	—
<i>Candida glabrata</i>	—	—	—	—	—	1	—	—	—	—
<i>Candida guilliermondii</i>	—	—	—	—	—	1	—	1	—	—
<i>Candida parapsilosis</i>	—	—	—	—	—	2	1	—	1	4
<i>Rhodotorula</i> sp.	—	—	—	—	—	—	2	—	—	—
<i>Rhodotorula mucilaginosa</i>	—	—	—	—	—	1	—	—	—	—
<i>Saccharomyces</i> sp.	—	—	—	—	—	—	—	—	1	2

^a Two neonatal ICUs at the same hospital, but in different buildings.

—, absence; n, no. of ants captured; ICU, adult intensive care unit; SC, surgery center; ICUneoI, neonatal intensive care unit I; ICUneoII, neonatal intensive care unit II; PW, pediatrics ward; HW, hematology ward; TW, transplant ward; SW, surgery ward; GW, gynecology ward.

sociotomy (colony fragmentation), and ability to easily change colonies from one place to another one (Chacón de Ulloa 2003, Passera and Aron 2005).

The semicritical areas in this study were highly prone to ant infestation, a pattern found in other studies (Moreira et al. 2005, Rodvalho et al. 2007). The substantially higher infestation observed in infirmaries could be related to a greater number of passersby, more available food for colonies, less rigorous hygiene, and lack of air conditioner (Bicho et al. 2007). In contrast, the infestation level was relatively low in critical areas, where only two ant species (*T. melanocephalum* and *P. longicornis*) were found, unlike the findings of studies conducted in other Brazilian hospitals, where neonatal wards and ICUs presented high levels of infestation (>40%) (Bueno and Campos-Farinha 1999). Air-conditioned environment and restricted access to those areas could explain the observed low infestation level.

Regarding ant distribution by period, the higher number of ants captured at night can be explained by

the presence of the genus *Camponotus*, characterized for its essentially nocturnal habits (Bueno and Campos-Farinha 1999), whereas the other genera found are more adapted to different periods of the day (Chacón de Ulloa 2003).

In this study, *T. melanocephalum* and the species of the genus *Pheidole* were carriers of airborne fungi and yeasts, whereas other genera only carried airborne fungi. Studies have shown that hospital infections can be caused by certain groups of fungi isolated from ants. This is the case of yeasts of the *Candida* genus and several species of airborne fungi, such as *Aspergillus*, *Cladosporium*, and *Penicillium* (Perdelli et al. 2006).

Among opportunistic fungi, the presence of yeasts from the *Candida* genus on ants is notable because it is the most important yeast genus implicated in human infections and is associated with almost 80% of the hospital fungal infections, representing the main cause of fungemia (Moretti 2007). Species from this genus can cause several diseases, such as arthritis, osteomyelitis, endocarditis, meningitis, and endophthalmitis

(Pfaller and Yu 2001). Other yeasts were isolated from the ants, such as *Rhodotorula* and *Saccharomyces*. This is an important observation, because these agents in contact with patients can cause infection, depending on some factors, such as the immune status of the patient (Ruiz-Esquide et al. 2002).

Therefore, the data suggest that ants can represent a risk to the patients, although the real involvement of these insects with transmission of illnesses is hard to demonstrate for ants, as well as other insects (Lemos et al. 2006). We should also point out the difficulty of differentiating the effects of fungi on ants and in hospital air, because the great majority of the fungi found in both the critical and semicritical areas were airborne species.

In summary, ants act as carriers of airborne fungi and yeasts and could therefore represent a potential risk to hospital patients. There is a need for better control of these insects in semicritical areas because of the presence of immunocompromised patients. In addition, these semicritical areas are important because they are the entrance to critical areas.

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