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Urban Ants and Transportation of Nosocomial Bacteria

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Formigas Urbanas e o Transporte de Bactérias Nosocomiais

RESUMO - Muitas espécies de formigas de comportamento sinantrópico que tiveram sucesso na dispersão em áreas urbanas podem causar problemas em hospitais ao atuarem como vetoras, transportando bactérias. Neste estudo, foram encontradas bactérias em formigas coletadas no Hospital de Clínicas da Universidade Federal de Uberlândia, no campus e em residências próximas. As formigas coletadas foram identificadas como *Tapinoma melanocephalum* (Fabricius) e *Camponotus vittatus* (Forel) (Hymenoptera: Formicidae) e as cepas bacterianas encontradas pertenciam ao grupo dos estafilococos coagulase positivos, estafilococos coagulase negativos e bacilos gram negativos, tendo sido encontradas cepas resistentes a antimicrobianos. O estudo de bactérias encontradas nas formigas e no ambiente mostrou que algumas formigas carreavam bactérias não isoladas do mesmo ambiente e com níveis de resistência mais elevados, evidenciando o potencial transmissor desses insetos.

PALAVRAS-CHAVE: Infecção hospitalar, *Tapinoma melanocephalum, Camponotus vittatus*, vetor

ABSTRACT - Many ant species displaying synanthropic behavior that have successfully dispersed in urban areas can cause problems in hospitals by acting as bacterial vectors. In this study, we encountered bacteria on ants collected at the Universidade Federal de Uberlândia hospital, in the campus and at households nearby. The ants were identified as *Tapinoma melanocephalum* (Fabricius) and *Camponotus vittatus* (Forel) (Hymenoptera: Formicidae) and the bacterial strains found here belong to the group of the coagulase-positive staphylococcus, coagulase-negative staphylococcus and gram negative bacilli, including antimicrobial drug-resistant strains. An investigation of the bacteria found in the ants and in the environment revealed that some ants carried non-isolated bacteria from the same environment and with high levels of resistance, evidencing the transmission potential of these insects.

KEY WORDS: Hospital infection, *melanocephalum, Camponotus vittatus*, vector

Many ant species that display synanthropic behavior have spread successfully in urban areas. The tramp species, for instance, have colonized regions whose climatic conditions were totally unfavorable (Suarez *et al*. 2001). Once established in the urban area, these ants can cause problems in offices, research institutes, vivariums, zoos, museums, libraries, telephone exchanges, homes and hospitals (Bueno & Campos-Farinha 1998, 1999; Marcolino *et al.* 2000). These problems involve nidification in electric installations and foraging in areas of risk, generating structural failures, contamination and disturbance to humans.

Several factors influence the occurrence of nosocomial infections. One of these is the presence of insects, among them the ants (Fowler *et al*. 1993, Bueno & Campos-Farinha 1999, Peçanha 2000). In hospital environments, ants can act as mechanical vectors, transporting bacteria, including those resistant to antimicrobials, and creating pathways of resistance dispersion in hospitals and in the external environment (Peçanha 2000). Bacteria that can be transported include gram negative bacilli (GNB), facultative aerobic or anaerobic bacteria, many of which are responsible for diseases of the gastrointestinal tract and of other organs. Since the population of this group is constituted of a high proportion of *Escherichia coli* (Siqueira 1995), the total coliform index is used to evaluate hygienic conditions, with high counts indicating post-processing contamination, inadequate cleaning and sanitization, ineffective heat treatments or multiplication during processing or storage (Tortora *et al*. 2000).

Also to be considered here are the gram positive cocci, members of the genus *Staphylococcus* (Tortora *et al*. 2000), which can be divided into two groups: coagulase-positive (CPS) and coagulase-negative (CNS) staphylococci. In the first group, *Staphylococcus aureus* is the most important pathogen associated with hospital infections, and also the main cause of infections in the community. With its high

virulence, it is able to become resistant to antimicrobials (Oliveira *et al.* 2000, Marin 2002). Among the coagulase-negative staphylococci, the methycillin resistant strains stand out for their frequent involvement in infections associated with foreign bodies and represent major resistance gene pools (Sanches *et al*. 2000), as observed in *S. epidermidis*, *S. hemolyticus*, *S. capitis*, *S. chromogenes*, *S. cohnii*, *S. equorum*, *S. felis*, *S. hominis*, *S. lentus*, *S. simulans*, *S. warneri* and *S. xylosus* (Becker *et al*. 2001).

In this study, we investigated the presence of three groups of bacteria (GNB, CPS and CNS) and their resistance profile in *Tapinoma melanocephalum* (Fabricius) and *Camponotus vittatus* (Forel) (Hymenoptera: Formicidae) ants collected at the Hospital das Clínicas, located on the campus of the Universidade Federal de Uberlândia, and at households nearby, with the purpose of evaluating the contaminating potential of the different ant genera.

Material and Methods

The ants were collected at the Hospital de Clínicas of the Universidade Federal de Uberlândia (HC-UFU) (in the sectors of Infectious Diseases, Burn Patients and Emergency Room), as well as at the 2E building, located 400 m from the hospital, and at homes adjacent to the campus.

The ants were collected between 6:30 p.m. and 8:30 p.m. and were identified according to Bolton (1995). Using sterilized tweezers, the ants were picked up and placed inside test tubes containing 2.5 ml of brain heart infusion (BHI - BIOBRÁS®) which were marked with the collection date, time and location. The contamination of the environment was ascertained by wiping the collection sites with a sterile swab.

In the laboratory, after removing the ants, the tubes were incubated for 36h to 42h at 37°C, after which they were subjected to depletion plaquing in Manitol Salt Agar (OXOID®) and MacConkey Agar (OXOID®) to isolate, respectively, *Staphylococcus* and gram negative bacilli. The seeded dishes were incubated for 24h at 37°C, after which the isolated colonies were subjected to gram staining.

The gram positive cocci were catalase and coagulase tested. The catalase and coagulase positive strains were considered coagulase-positive staphylococci (CPS), while catalase-positive and coagulase-negative strains were considered coagulase-negative staphylococci (CNS). Both were evaluated in terms of their antimicrobial resistance profile. The isolated gram negative bacilli (GNB) were also evaluated for antimicrobial sensitivity.

To verify the presence of total coliforms (TC) and thermotolerant coliforms (fecal coliforms - FC), brilliant green lactose bile broth 2% (DIFCO®) and EC broth (DIFCO®) culture mediums were used with Durham tubes inverted into them. About 0.5 ml of the culture grown in BHI was added to the test tubes containing the aforementioned mediums. The tubes containing brilliant green broth were incubated at 37°C for 24h, while the tubes containing EC broth were incubated in a double boiler at 44.5°C for 24h. The tubes presenting turbidity and the presence of gas (bubbles inside the Durham tubes) were considered positive.

The drug sensitivity test was carried out by means of the disc diffusion technique, as described by Kirby-Bauer (Bauer *et al*. 1966). The following antimicrobials were tested for staphylococcus strains: cephalotine (30 μg), oxacillin (1 μg), penicillin G (10 UI), tetracycline (30 μg) and vancomycin (30 μg). Ampycillin (10 μg), cephalotine (30 μg), ciprofloxacine (5 μg) and sulphazotrin (25 μg) were tested for the GNB. These antimicrobials were chosen because they represent the main classes of drugs utilized. The halos were measured with a pachymeter and the results interpreted according to the manufacturer's instructions, classifying the strains into the categories of resistant, intermediary or sensitive, as established by the National Committee for Clinical Laboratory Standards (Nccls 2000). *S. aureus* ATCC25923 and *E. coli* ATCC25922 were used as control strains in the susceptibility tests.

Results and Discussion

Table 1 presents the data from the samples collected at the Hospital das Clínicas, from the campus and from adjacent homes, as well as all the other results. *Camponotus* was found at all the collection sites, and was the only genus present in the hospital's Infectious Diseases and Burn Patients sectors. The latter finding is relevant because of the high risk factor at these sites, which house debilitated and immunodepressive patients. *Tapinoma* was found in the Emergency Room, on campus and in the adjacent homes. The two genera collected at the hospital are among those most commonly found in other hospitals in Brazil, but apart from these, other genera have been found (Bueno & Campos-Farinha 1998, Peçanha 2000).

A total of 30 individuals were collected, of which 16 were identified as *C. vittatus* and 14 as *T. melanocephalum*. Ten of these ants (33.3%) were contaminated with bacteria. Of these 10 ants, 5 (16.7%) were collected at the hospital, 4 (13.3%) on campus and 1 (3.3%) in adjacent homes. Fifteen (57.7%) of the 26 specimens collected in the environment were contaminated, i.e., 7 (27.0%) from the hospital, 5 (19.2%) from the laboratory and 3 (11.5%) from residences.

Moreover, CPS and GNB were isolated both from ants and from the environment, while CNS were only isolated from ants, suggesting that ants may be responsible for carrying and distributing CNS both inside and outside the hospital environment. Of the six bacterial strains isolated at the HC-UFU, only 1 (16. 7%) belonged to this group. This contamination rate is lower than that found by Peçanha in 2000 (21.4%). The large majority of the strains isolated from swabs corresponded to CPS (81.3%). In 56.3% of the cases, these strains were isolated only from the environment and were not found in ant samples.

Out of all the strains isolated from ants found in the hospital, 58.3% were CPS, a very high rate when compared with the 8.3% found by Peçanha (2000) and Moreira *et al*. (2005). It is worth noting that both authors isolated only *S. aureus*. Of the samples obtained from swabs at the hospital, 85.7% of the isolated strains belonged to this group. In contrast, Peçanha (2000) found only 3.3% of contamination by *S. aureus* (CPS) from swabs.

Table 1. Collection sites, samples and resistance profiles of the strains isolated from ants and from the environment.

	Sample[1]	Site	Ant species	Bacterium Ant	Bacterium Environment	Antimicrobial resistance
Hospital de Clínicas	1	ID	*C. vittatus*	--	--	
	2	ID	*C. vittatus*	--	--	
	3	ID	*C. vittatus*	--	--	
	4	ID	*C. vittatus*	CPS	CPS[2]	iO, P / P
	5	ID	*C. vittatus*	CPS		T
	6	B	*C. vittatus*	CPS	--	sensitive
	7	B	*C. vittatus*	--	--	
	8	B	*C. vittatus*	--	--	
	9	ER	*T. melanocephalum*	--	CPS	T, P
	10	ER	*T. melanocephalum*	--	TC	sensitive
	11	ER	*C. vittatus*	--	--	
	12	ER	*C. vittatus*	CNS, GNB	CPS	P,iT/A,Ce,iCi/Ce,O,P
	13	ER	*T. melanocephalum*	--	--	
	14	ER	*T. melanocephalum*	--	CPS	P
	15	ER	*T. melanocephalum*	--	CPS	P
	16	ER	*T. melanocephalum*	--	--	
	17	ER	*T. melanocephalum*	CPS	CPS	P / T
Campus	18	2E	*T. melanocephalum*	--	--	
	19	2E	*T. melanocephalum*	--	--	
	20	2E	*T. melanocephalum*	CPS, GNB	--	Ce,O,P,iT,V / iA
	21	2E	*T. melanocephalum*	CNS	CPS, TC	P / P / sensitive
	22	2E	*C. vittatus*	CPS	CPS	P / iP
	23	2E	*C. vittatus*	--	CPS	P
	24	2E	*T. melanocephalum*	CPS	CPS	Ce,O,P,V/sensitive
	25	2E	*T. melanocephalum*	--	CPS	P
Adjacencies	26	H	*T. melanocephalum*	--	CPS	O, P
	27	H	*C. vittatus*	--	--	
	28	H	*C. vittatus*	--	--	
	29	H	*C. vittatus*	--	CPS	P
	30	H	*C. vittatus*	GNB	GNB	A, Ce/sensitive
Total	30			12	16	

[1]SAMPLE: number of the sample; Site: (ID) Infectious diseases, (B) Burn patients, (ER) Emergency room, (2E) 2E building, (H) Homes; (CPS) coagulase-positive staphylococci, (CNS) coagulase-negative staphylococci, (GNB) gram-negative bacilli, (TC) total coliforms; Resistance: (A) ampycillin, (Ce) cephalotine, (Ci) ciprofloxacine, (O) oxacillin, (P) penicillin, (T) tetracycline, (V) vancomycin, and (i) Intermediary resistance.
[2]Environmental contamination was found by using the same swab for the two ants.

Out of the total of GNB found, 75% came from ants and only one sample (25%) was obtained from the environment (homes). The GNB strains isolated from ants from the hospital and campus showed no correspondence with the respective swabs. As in the case of the CNS, this suggests that these insects may be responsible for carrying and distributing GNB bacteria both inside and outside the hospital environment. The 16.7% contamination rate of ants by such bacteria in

the hospital was lower than Moreira *et al*. (2005) found and the 66.7% found by Peçanha (2000). TC were found on two occasions in samples collected from the environment, hospital and laboratory. However, FC were not found.

Among the ant species collected, *C. vittatus* presented the highest level of contamination (37.5%), followed by *T. melanocephalum* (28.6%), both species carried the three groups of bacteria (GNB, CPS, CNS). Possibly *T. melanocephalum* is less contaminated due to its preference for clean places. Most of the seven strains of *C. vittatus* isolated (57.1%) corresponded to CPS, followed by GNB (28.6%) and CNS (14.3%). We also found that, of the six contaminated ants, four (66. 7%) were collected in the hospital, a lower rate than that found by Peçanha (2000) (90.0%), although this author also found ants of the *Camponotus atriceps* (Smith) species.

The *T. melanocephalum* individuals yielded five bacterial strains, three belonging to the CPS group, one GNB and one CNS. Those bacteria were also found on ants of the same species in Campo dos Goytacazes (Moreira *et al.* 2005). Most of the contaminated ants belonging to this genus (75.0%) were collected on the campus, while only one, carrying CPS, was found in the hospital. The ant collected in sampling number 20, on campus, carried both CPS and GNB, although these bacteria were absent from the corresponding environments.

The results obtained indicate that the ant species *T. melanocephalum* and *C. vittatus* are major bacterial vectors in hospital environments. The former, albeit displaying a low contamination rate (28.8%), may become a serious vector since its presence is abundant in the environment. Moreover, this species is difficult to control because it is polygenic, presents unicolonial populations, high dispersion and reproduction rates, absence of nuptial flight, and can present sociotomy (fragmentation of the colony) under environmental pressure. The species *C. vittatus* is important for its dissemination, abundance and for presenting the highest rate of contamination (37.5%), even carrying bacteria not found in the environment. This rate is lower than that reported by previous studies, in which was found a 75% association between *C. atriceps* and *S. aureus* (data not published).

An overall analysis indicated that the bacterial strains isolated from the Hospital de Clínicas showed resistance to a larger number of antimicrobials. However, considering the CPS isolated from ants, we found greater resistance among the strains isolated from the campus, including resistance to vancomycin of exclusive hospital use, the last effective form of treatment of serious infections caused by methycillin-resistant *S. aureus* (Mendoza *et al.* 2000, Oliveira *et al*. 2001, Goldrick 2002, Marin 2002). The two strains presenting resistance to this antibiotic were collected from two individuals of *T. melanocephalum* (numbers 20 and 24), but this level of resistance was not found in strains isolated from the respective environments, which reinforces the importance of this species as a major bacterial vector. Another aggravating factor is that the resistance to vancomycin was accompanied by multiple resistance to other antimicrobials, increasing the potential risks posed by *T. melanocephalum*. Although vancomycin and oxacillin-resistant strains were found, no tests were conducted to confirm this resistance. The GNB isolated from the hospital displayed a higher resistance profile than those isolated from other sites. Moreira *et al*. (2005) also investigated the presence of antimicrobial-resistant bacteria carried by ants isolated from hospitals and among the bacteria isolates, some were considered multiresistant, including genera *Acinetobacter, Streptococcus, Gemella* and *Klebsiella*.

It can therefore be concluded that ants are important vectors of bacteria, presenting a high level of contamination, and can be considered important vectors in hospital environments. Furthermore, the bacteria carried by ants show higher levels of resistance than the bacteria isolated from the environment, indicating that ants are disseminators of bacterial resistance.

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