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Microbiota Associated with Tramp Ants in a Brazilian University Hospital

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Microbiota Associada às Formigas Urbanas em um Hospital Universitário Brasileiro

RESUMO - O objetivo deste estudo foi conhecer a fauna de formigas existente no Hospital Universitário da Universidade Federal do Triângulo Mineiro, Uberaba, MG, bem como as espécies de microrganismos transportadas por elas, além de identificar o padrão de resistência destes microrganismos a antimicrobianos. Para atrair as formigas, utilizaram-se tubos estéreis contendo mel. Após período de exposição de 3h, os mesmos foram tampados. Os tubos que atraíram formigas foram considerados o grupo teste e os que não atraíram o controle. Apenas *Tapinoma melanocephalum* (Fabricius) foi amostrada. O isolamento de microrganismos dos exemplares de *T. melanocephalum* apontou 60 espécies, entre as quais sete eram bacilos Gram positivo, 14 bacilos Gram negativo, 22 cocos Gram positivo e 17 fungos filamentosos. *Pseudomonas*, *Staphylococcus* e *Streptococcus* do grupo D foram os microrganismos que apresentaram maior resistência aos antibióticos. As formigas devem ser consideradas um importante vetor de infecções, pois são carreadoras de microrganismos, levando-os na superfície de seu corpo para materiais estéreis, equipamentos e alimentos não-contaminados. Não é possível definir o papel exato das formigas em infecções nosocomiais. No entanto, este deve ser melhor avaliado e atenção especial deve ser dada pelas comissões de Controle de Infecções Hospitalares.

PALAVRAS-CHAVE: Infecção hospitalar, microrganismo patogênico, resistência antimicrobiana, distribuição

ABSTRACT - Our aim was to study the fauna of ants in the Hospital Universitário of the Universidade Federal do Triângulo Mineiro, municipality of Uberaba, Minas Gerais State, Brazil, as well as to identify the microorganisms the ants carry and their patterns of resistance to antibiotics. Sterile tubes (traps) containing honey were used to attract the ants. Traps were exposed for 3h, and those which attracted ants were considered the test group, while the ones that did not attract the insects constituted the control group. Only the ant species *Tapinoma melanocephalum* (Fabricius) was sampled. Sixty microorganisms were isolated from the sampled ants, including seven Gram-positive bacilli, 14 Gram-negative bacilli, 22 Gram-positive cocci and 17 filamentous fungi. *Pseudomonas*, *Staphylococcus* and Group D *Streptococcus* were the microorganisms with the highest resistance to the tested antibiotics. The ants should be considered an important vector of infections as they carry several pathogenic microorganisms, spreading them on the surface of sterile materials, equipments and uncontaminated food. It is impossible to define the exact role of ants in nosocomial infections at this moment; however, this issue must be better studied and special attention must be given by the commissions of Nosocomial Infection Control.

KEY WORDS: Hospital infection, pathogenic microorganism, antimicrobial resistance, distribution

Several reports have alerted to the specific role of insects in the transport of associated microorganisms in the hospital environment, which favors nosocomial infections (Beatson 1972, Eicheler 1990, Sramova *et al* 1992, Sawicka 1993).

Ants not always were considered noxious insects, although an eventual role in the transport of microorganisms has been investigated since the seventies. More often, ants cause problems if they appear in great numbers, and not because they can act as mechanical vectors of pathogenic

microorganisms (Ipinza-Regla *et al* 1981).

In Brazil, this kind of study is relatively recent. In 1993, 14 ant species were described associated to hospitals in the State of São Paulo (Fowler *et al* 1993). The predominance of particular ant species varied among the different sampled institutions, but the risk of an eventual association between these insects and the hospital infections was already emphasized.

Factors that may favor the presence of ants in hospitals include the building architecture, proximity to residences, occurrence of packages of medicines containing nests of ants, circulation of people with clothes and/or objects carrying ants, in addition to food remains which constitute an extra attractive (Zarzuela *et al* 2002).

Our objective here was to study the existent fauna of ants in the Hospital Universitário of the Universidade Federal do Triângulo Mineiro, and to disclose the species of microorganisms they carry. We also aimed at identifying the patterns of antimicrobial resistance developed by these microorganisms.

Material and Methods

The study was accomplished at the Intensive Care Units (ICUs) and surgical center from the Hospital of UFTM, in Uberaba, MG. Samples were collected monthly, from May 2005 to May 2006.

Collection and identification. Ten sterilized test tubes containing honey were left in specific sites of the hospital by 3h in order to attract ants. Afterwards, the tubes were closed and sent to the microbiology laboratory of the Hospital. The tubes which attracted ants constituted the test group (TG), and those tubes without ants were considered the control group (please see Discussion) (CG).

In the laboratory, the ants were immersed in tubes with thioglycolate broth, agitated and incubated at $36 \pm 1^\circ\text{C}$ for 48h. Aliquots of the thioglycolate broth were plated onto agar-blood for isolation, and again incubated at $36 \pm 1^\circ\text{C}$ for 24-48h. The Gram staining method was utilized for every plate showing bacterial growth. In case of fungi growth, they were simply classified as yeast-like or filamentous fungi.

Colonies of Gram-positive cocci were characterized according to their morphology, by the method of Gram, and the catalase and coagulase proofs (Koneman *et al* 2001). Disks with novobiocin were used to distinguish among the *Staphylococcus cepa*. To distinguish *Streptococci*, in addition to detection of hemolysis, tests with bile-esculin, 6.5% sodium chloride and optochin were utilized.

Antimicrobial susceptibility. Bacteria identified in the TG were submitted to the disk diffusion test for determination of the bacterial susceptibility to the tested antimicrobials, utilizing the Mueller Hinton broth, following the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI 2005).

The antibiotics to test the Gram-negative bacilli included: G-penicillin, chloranfenicol, gentamicin, amikacin, aztreonam, cefalotin, levofloxacin and ciprofloxacin. The antibiotics to test the Gram-positive cocci were: oxacillin,

G-penicillin, chloranfenicol, gentamicin, amikacin, vancomycin, cefalotin and ciprofloxacin. As a whole, the enrolled antibiotics from the Laborclin trademark were chosen because they are frequently used to treat patients in the Hospital of UFTM. The following strains were used for control purposes: ATCC *Escherichia coli* 25922, ATCC *Enterococcus faecalis* 29212, ATCC *Staphylococcus aureus* 25293 and ATCC *Klebsiella pneumoniae* 700603.

The profiles of resistance of the samples collected in ants were compared with the database of Commission of Nosocomial Infection Control - CNIC, of the Hospital in the UFTM from May to October of 2005.

The frequency of each microorganism occurrence, both in TG and CG were subjected to by ANOVA considering the parametric distribution, using the program MINITAB® according to Draper & Smith (1981) at $P \leq 0.05$. The descriptive analysis of the sensitivity profile of bacteria isolated from ants was compared to those isolated from patients in the CNIC.

Results

Twelve collections from the intrahospital mirmecofauna were accomplished to identify the associated microbiota in four different sites from the Hospital of UFTM: ICU (adults), 5; ICU (pediatrics), 4; ICU (dressing room), 1; Surgical center (dining hall), 1. Only *Tapinoma melanocephalum* (Fabricius) was collected during the period of this study.

The isolation of microorganisms from the *T. melanocephalum* samples revealed 60 species, including seven Gram-positive bacilli, 14 Gram-negative bacilli, 22 Gram-positive cocci and 17 filamentous fungi. Only seven microorganisms were isolated from the control tubes, two *Bacillus* sp. and five coagulase-negative *Staphylococci* (Table 1).

Table 1 Frequency distribution, by morph species, of the microorganisms identified from ants collected in the Hospital of UFTM, between May of 2005 and May of 2006.

Microorganisms	Frequency text group	%	Frequency control group	%
<i>Bacillus</i> sp.	7	11.8	2	28.6
<i>Burkholderia cepacia</i>	3	5	0	0
Coagulase-negative <i>Staphylococci</i>	14	23.7	5	71.4
<i>Enterobacter aerogenes</i>	2	3.4	0	0
Filamentous fungi	17	28.8	0	0
Group D <i>Streptococci</i>	5	8.5	0	0
<i>Hafnia alvei</i>	3	5	0	0
<i>Pseudomonas</i> sp.	5	8.5	0	0
<i>Staphylococcus saprophyticus</i>	3	3.4	0	0
Total	59	100	7	100

Table 2 Resistance (%) of the microorganisms isolated from ants collected in the Hospital of UFTM, between May 2005 and May 2006, to selected antibiotics.

Microorganisms	Gn	Chl	Am	Cep	Cip	Lev	Az	Im	Van	Ox	Pen	N
<i>B. cepacia</i> ¹	0	67	67	100	0	0	67	33	-	-	-	3
Coagulase (-) <i>Staphylococci</i> ¹	0	0	0	50	0	-	-	-	0	50	50	14
Coagulase (-) <i>Staphylococci</i> ²	0	0	0	0	0	-	-	-	0	0	60	5
<i>E. aerogenes</i> ¹	0	0	0	0	0	0	0	0	-	-	-	2
Group D <i>Streptococci</i> ¹	80	40	80	80	40	-	-	-	0	80	80	5
<i>Hafnia alvei</i> ¹	0	33	0	0	0	0	0	0	-	-	-	3
<i>Pseudomonas</i> sp. ¹	20	40	20	20	20	20	40	40	-	-	-	5
<i>Staphylococcus saprophyticus</i> ¹	0	33	0	66.6	0	-	-	-	0	66.6	66.6	3

Gn: Gentamicin; Chl: chloranfenicol; Am: amikacin; Cep: cephalotin; Cip: ciprofloxacin; Lev: levofloxacin; Az: aztreonam; Im: imipenem; Van: vancomycin; Ox: oxacillin; Pen: penicillin; N: number of samples

¹test group; ²control group

Filamentous fungi were the microorganisms that presented the highest frequency (28.8%), followed by coagulase-negative *Staphylococcus* (23.7%), Gram-positive bacilli (11.8%) and *Pseudomonas* sp. (8.5%) (Table 1). Gram-positive bacilli ($P = 0.030$), *Pseudomonas* sp. ($P = 0.009$), *Burkholderia cepacia* ($P = 0.045$), *Hafnia alvei* ($P = 0.045$), Group D *Streptococcus* ($P = 0.009$), *Staphylococcus* sp. ($P = 0.001$) and filamentous fungi ($P = 0.000$) occurred at different frequencies between test and control groups (Table 1).

Burkholderia cepacia showed 100% resistance to cephalotin and 67% resistance to chloranfenicol, amikacin and aztreonam (Table 2). In the resistance profiles obtained by the CNIC in the Hospital of UFTM from May to October of 2005 (Table 3), there was no report of infections by *B. cepacia*. *Pseudomonas* sp. presented 40% resistance to chloranfenicol, aztreonam and imipenem (Table 2); when compared to the data from CNIC, the percentages of resistance were 47.3%, 50% and 31.6%, respectively (Table 3). Among the Gram-positive cocci, 60% of the coagulase-negative *Staphylococcus* were resistant to oxacillin; hence, they were also considered resistant to other beta-lactamic agents (penicillin and cefalotin) (CLSI, 2005). Similar profiles were observed among agents isolated by the CNIC in the Hospital of UFTM; 80% of the group D *Streptococci* were resistant to gentamicin, amikacin, cefalotin, oxacillin and penicillin. In relation to the group D *Streptococci*, the CNIC has found

much lower rates of resistance to gentamicin, amikacin and penicillin: 51.7%, 62% and 34.5%, respectively.

Discussion

Similar to many other reports, the presence of ants was observed in the Hospital of UFTM, which is in accordance with the developing countries reality (Beatson 1972, Edwards & Backer 1981, Ipinza-Regla *et al* 1981, Sawicka 1993). The frequent use of these areas, in addition to cleaning and sanitation procedures often promote a gradual deterioration of the most fragile materials, especially in the covered openings and junctions of the walls, floors and ceilings. Even small crevices constitute ideal shelters for insects, in special for ants that usually live in urban structures (Schuler 1999).

Similar to the study of Moreira *et al* (2005), the control group of the present work was the ambient air. The purpose was to avoid eventual bias represented by microorganisms directly derived from the contaminated hospital air.

The ant species most commonly found in hospitals in the state of São Paulo were *T. melanocephalum* and *Paratrechina longicornis* (Latreille), but the first one was more prevalent (Fowler *et al* 1993). The identification of the microbiota from the collected ants was also done and *Staphylococcus*, *Serratia*, *Klebsiella*, *Acinetobacter*, *Enterobacter*, *Candida*

Table 3 Resistance (%) of microorganisms isolated from patients of the Hospital of UFTM, between May and October of 2005, to selected antibiotics.

Microorganisms	Gn	Chl	Am	Cep	Cip	Lev	Az	Im	Van	Ox	Pen	N
Coagulase (-) <i>Staphylococci</i>	45.2	23	24.6	64.7	46	-	-	-	0.8	64.3	80.5	29
<i>E. aerogenes</i>	28.6	35.7	17.8	96.4	25	28.6	28.6	3.6	-	-	-	19
group D <i>Streptococci</i>	51.7	51.7	62	-	55.1	-	-	-	6.9	-	34.5	
<i>Pseudomonas</i> sp.	42.1	47.3	42.1	76.3	18.4	21	50	31.6	-	-	-	28
<i>S. saprophyticus</i>	15.8	15.8	5.2	63.1	21	-	-	-	0	73.7	42.1	25.2

Gn: Gentamicin; Chl: chloranfenicol; Am: amikacin; Cep: cephalotin; Cip: ciprofloxacin; Lev: levofloxacin; Az: aztreonam; Im: imipenem; Van: vancomycin; Ox: oxacillin; Pen: penicillin; N: number of samples

and *Enterococcus* were isolated.

Although *T. melanocephalum* was the sole finding in the present study, other species may be found in our hospital, since the collections were limited to four specific areas. Moreover, in another study performed in our hospital from 2001 to 2005, samples of *T. melanocephalum*; *Pheidole sp.* and *Paratrechina longicornis* were captured (Costa et al 2006). The observed reduction in the number of ant species may be associated with the recent reforms that were made in the areas of the ICUs. Furthermore, while Costa et al (2006) collected ants in several environments; the present study was restricted to the ICUs and the surgical center. It is worthwhile to note that *T. melanocephalum* was the least frequent among seven ant species identified in two hospitals from the state of Santa Catarina (Lise et al 2006). *Taponoma melanocephalum* is commonly called ghost ant and is included in the group of tramp ants, which live in intimate association with humans (Campos-Farinha & Bueno 2003).

In this study, the ICUs and the surgical center were the chosen areas for collection because of the high number of patients under risk of hospital infection due to their low immunity. Moreover, a high index of ant infestation has been described in ICUs (Bueno & Campos-Farinha 1998).

In the state of Rio de Janeiro, four species of ants were described as carriers of hospital bacteria resistant to antimicrobials, being *T. melanocephalum* the most frequent (63.1%) (Moreira et al 2005). The enrolled bacteria included *Bacillus spp.*, *Enterobacter sp.* and *Staphylococcus sp.*, with a high frequency of coagulase-negative *Staphylococcus* resistant to oxacillin.

Coagulase-negative *Staphylococci* can play important role in immunosuppressed patients (Minto et al 1999). In the present study the bacteria were isolated in hospital environments with a high number of immunosuppressed patients; therefore, all the *Staphylococcus* that we found must be considered pathogenic. Coagulase-negative *Staphylococci* are among the main causes of hospital bacteremia, in special affecting patients from ICUs (Cunha et al 2002).

Strains of methicillin-resistant *Staphylococcus* are often described (Torres et al 2001); moreover, the ability of *S. epidermidis* to form biofilms may contribute to the origin of nosocomial infections involving hospital instruments (Dunne et al 1993).

Gram-negative bacilli, including *Pseudomonas sp.* and *Enterobacter sp.* represent a great risk in the hospital infections (ANVISA 2004). *Pseudomonas sp.* is an opportunistic pathogen, found in children submitted to mechanic ventilation in ICUs (Miranda et al 1999, Murray et al 2000). The wide range of their antibiotic resistance patterns indicate that strains of these pathogens are continuously selected (Banderó Filho et al 2006).

Hospital infections remain a common and serious unsolved challenge, which causes high morbidity and mortality rates. This condition increases the cost of treatment and the time of hospitalization, and constitutes a constant threat to the spread of multiresistant bacterial pathogens (Far et al 2001).

Although the role of ants in the hospital infections is not entirely cleared, there are sufficient data to support this possibility and the associated risks. Therefore, this matter should be better considered and settled by the commissions

of Hospital Infection Control. Moreover, further studies must be performed to verify possible correlations between the microbial strains isolated from the ants and the strains isolated from patients with hospital infections acquired in the occasion of the ant's collections.

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