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Ants as Carriers of Antibiotic-Resistant Bacteria in Hospitals

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Formigas Como Agentes Carreadores de Bactérias Resistentes em Hospitais

RESUMO - A presença de bactérias resistentes a antimicrobianos e carreadas por formigas isoladas em hospitais foi investigada em Campos dos Goytacazes, RJ. Três instituições de saúde foram visitadas nos períodos de 2001 a 2002 e amostras foram coletadas em áreas de cuidados médicos consideradas críticas baseando-se em critérios de maior risco para a aquisição de infecções por pacientes hospitalizados. Quatro espécies de formigas foram identificadas, *Tapinoma melanocephalum* (Fabricius) (63,1%), *Paratrechina longicornis* (Latreille) (21,1%), *Monomorium pharaonis* (L.) (10,5%) e *Solenopsis saevissima* (S. Smith) (5,3%) como carreadoras de 21 morfoespécies de bactérias. Os testes de identificação bacteriana e de perfil de resistência antimicrobiana foram realizados por meio de kits padronizados e avaliados por método automatizado. Entre as bactérias isoladas, algumas foram consideradas multirresistentes, incluindo o gênero *Acinetobacter*, *Streptococcus*, *Gemella*, e *Klebsiella*. Para análise antimicrobiana do gênero *Enterobacter* foi utilizado o método padrão de difusão em disco. Os resultados sugerem que existem riscos para pacientes que procuram por assistência de saúde nos hospitais estudados e a ocorrência de bactérias emergentes em hospitais carreadas por formigas devem ser consideradas.

PALAVRAS-CHAVE: Infecçção nosocomial, antimicrobiano, controle, formiga urbana

ABSTRACT - The presence of antimicrobial-resistant bacteria and carried by ants isolated from hospitals was investigated in Campos dos Goytacazes, RJ, Brazil. Three health institutions were visited from 2001 to 2002 and samples were collected within critical areas of medical care based on criteria of greater risk of patient hospital acquired-infection. Four ant species were identified, *Tapinoma melanocephalum* (Fabricius) (63.1%), *Paratrechina longicornis* (Latreille) (21.1%), *Monomorium pharaonis* (L.) (10.5%), and *Solenopsis saevissima* (S. Smith) (5.3%) carrying 21 species of bacteria. The tests for bacteria species identification and antimicrobial-resistance profile were carried out by using standardized kits and evaluated by automated equipment. Among the bacteria isolates, some were considered multiresistant isolates, including genera *Acinetobacter, Streptococcus, Gemella*, and *Klebsiella*. For *Enterobacter* antibacterial analysis the disk diffusion standard method was used. The results suggest that there are risks for patients which seek for health assistance in the hospitals studied, and the presence of emerging bacteria isolates in hospital carried by ants must be considered.

KEY WORDS: Nosocomial infection, antimicrobial, control, urban ant

Ants are often a serious problem in Brazilian hospitals, since they may be found in large numbers and are difficult to control. The occurrence and abundance of ants are frequently high because of the structural characteristics of the buildings, such as easy access to the outside environment, and proximity to houses. Inadequate management practices

are also important factors: improper storage of medication, intense traffic of people and presence of food aggravate the ant problem (Fowler *et al* 1993, 1995; Bueno & Campos Farinha 1999a; Santos *et al*. 2001; Zarzuela *et al*. 2002). Ants colonize hospital equipment, invade sensitive areas such as intensive care units and become a nuisance to staff and

patients. According to some authors many factors contribute to ants colonization in hospital environment, such as sterilizing products, and personal stuffs brought by visitors to patients could play a role in transportation of ants inside hospital facilities (Santos 2001, Bueno & Campos Farinha 1999b). The colonization also occur mainly due to ant size, the smallest ones may vary from 1.3 mm to 2.2 mm in size (Silva & Loeck 1999). Added to the fact is the availability of food and water supply in a cozy place to nest formation (Bueno & Campos Farinha 1999a,b). Factors that also help to multiplication of this pest are polyginy, frequent moving, high capacity of reproduction.

The adaptation to a vast diet has made ants classification range from omnivore species, feeding whatever they find, with meals that include animal, vegetal or even sweet; some are carnivores feeding from live to dead animals, while others use nectar or juice of plants or fungi as nourish supplies (Fowler et al 1991, Bueno & Campos Farinha 1999a).

The eradication of urban ants became a complex task, mainly because nest localization, occurrence of many nests in the same area, constant re-infestation and adaptation of some species to human habits, besides colony fragmentation (Bueno & Campos Farinha 1999 a,b). However, some factors must be taken into account when the objective is to control ants in hospitals, as follows: keep place clean and food storage appropriately, avoiding empty space between wall revetments and plant pots which may contribute to local infestation (Santos 2001). Its also noteworthy to mention the practice of using a mixing of Vaseline added to hydration baby oil or double-face tape around supports to avoid ants to climbing beds, cribs and incubators; keeping all mobile furniture distant from walls and mechanic destruction of nests which consists the most efficient method of eradication (Bueno & Campos Farinha 1999a,b).

Moreover, ant/bacteria associations have been found in several hospitals, raising concerns about the possible role of ants as disease vectors (Chadee & Le Maitre 1990, Fontana et al. 2001). This is a potentially serious issue, especially considering that resistant bacteria strains have been found in ants collected in Brazilian hospitals (Peçanha 2000, Fontana et al. 2001). Patients in some hospital areas such as trauma, surgery, intensive care and burns units are under particular risk, since ants may be in direct contact with wounds, bandages and hospital apparatus (Beatson 1972, Fowler et al. 1993).

Bacteria are the most important agents of nosocomial infections, and *Staphylococcus* spp., *Pseudomonas* spp. and *Enterobacter* spp. are some of the microorganisms most often detected (Lowy 1998, Arruda *et al.* 1999, Levin *et al.* 1999, Pellegrino *et al.* 2002). Our objective was to identify the ant species present in three of the largest hospitals in Campos dos Goytacazes, Rio de Janeiro State, Brazil, identify the bacteria associated with these ants and evaluate their resistance levels to antibiotics.

Materials and Methods

From November 2001 to April 2002, 38 samples of ants were collected at approximately weekly intervals from

critical points in three hospitals, thereafter referred to as A, B and C, in Campos dos Goytacazes. The selection of critical points was based on the criteria of greater risk of patient infection (Beatson 1972, Fowler et al. 1993) and higher incidence of ants, according to the medical staff. The following critical points were selected from Infirmaries (INF) and Intensive Care Units (ICU) in each hospital. A: pediatric ICU, general INF, and contagious diseases INF; B: adult ICU, post-surgery INF, pediatric INF, and gynecological INF; C: pediatric ICU, pediatric INF, trauma INF, and cardiac INF. Each sample consisted of 10 individuals from each morphological species, which were collected with sterilized forceps and transferred to vials containing 10 ml of Brain Heart Infusion (BHI) (Merk, Germany). For each sample collected, another vial with the same volume of medium was opened and closed as a control for possible media contamination. All samples were collected between 2.00 pm and 4.00 pm. For species identification, 20 individuals from each morphological species were collected and transferred to vials with 70% alcohol. Part of the collection was deposited in the Museu de Entomologia of the Universidade Estadual do Norte Fluminense Darcy Ribeiro, and part was deposited in the reference collection of Instituto Biológico de São Paulo, SP, Brazil.

Samples were incubated at 35°C for 48h. After this period, vials without visible bacteria growth were discarded; otherwise they were submitted to serial dilution, and one 100 µl sub-sample was added to petri dishes, each with one of the following growth media: S-S Agar (Merck, Germany), Azide Blood Agar Base (Oxoid, UK), Mannitol Salt Agar (Merck, Germany), MacConkey Agar (Merck, Germany), and Plate Count Agar (Merck, Germany).

From preliminary evaluations, 10⁻⁵ was shown the most suitable dilution regarding the purity of Colony Forming Units (CFUs) after incubation in petri dishes. Petri dishes, each with 100 µl of sterile water, were used as controls. All dishes were incubated at 35°C for 24h initially, and for an additional 24h if necessary. Bacteria samples were added to vials containing 5 ml of BHI, and these vials were incubated at 35°C for 16h to 24h. Subsamples (850 µl) from those media with visible bacteria growth were added to 150 µl of sterilized glycerol. The mixture was stirred for 30 s, subsequently frozen in liquid nitrogen and stored at -80°C. The isolates were reactivated in their respective media vials for macroscopic and microscopic visual evaluations, as well as for Gramstain testing. All Gram-positive bacteria were submitted to oxidase and catalase tests with a BBL Dryslide kit (Difco, USA) and 10% peroxide, respectively. All catalase-positive and Gram-positive cocci were tested for deoxyribonuclease activity on DNase agar (Merck, Germany). For the agglutination test, a Slidex Staph Plus kit (BioMérieux, France) was utilized. The isolates were also submitted to the free coagulase test in tubes containing rabbit plasma EDTA (Laborclin, Brazil). The isolates were identified with the aid of the following kits according to the manufacturers' instructions: Rapid ID 32 Staphylococcus, ID 32 Streptococcus, ID 32E Enterobacteriaceae. All kit reactions were read with the

MiniApi automated system (bioMérieux, Italy) and those with readings above the 85% identification profile were considered in this study. Furthermore, bacteria classified as *Streptococcus*, *Gemella*, *Staphylococcus*, as well as Enterobactericeae, were selected according to their nosocomial, medical and pathogenic profiles and submitted to antimicrobial susceptibility tests. These tests were performed with the following kits from bioMéuriex (France): ATB Staph (*Staphylococcus* spp.), ATB Strep (*Streptococcus* spp. and *Gemella* spp.), and ATB GN-5 (*Enterobacteriaceae* and other Gram-negative bacteria). Gram-positive *Enterobacter faecalis* and *E. faecium* were tested for resistance and susceptibility against twelve pathern antibiotic drugs by the disk diffusion standard method (Laborclin, Brazil).

Results

Four ant species were collected in three hospitals: *Tapinoma melanocephalum* (Fabricius) (63.1%), *Paratrechina longicornis* (Latraille) (21.1%), *Monomorium pharaonis* (L.) (10.5%), and *Solenopsis saevissima* (F. Smith) (5.3%). All species of ants were contaminated with several bacteria species (Table 1), however those present on *S. saevissima* could not be identified, as any attempt to reactivate stocked samples resulted negative. None of the control tubes presented bacteria growth.

The Streptococcus acidominimus isolate collected from P. longicornis in hospital A was resistant to penicillin pneumococci, penicillin streptococci, amoxicillin pneumococci, cefotaxim pneumococci, cefotaxim estreptococci, erythromicin, quinopristindalfopristin, clindamycin, tetracyclin, clorafenicol, vancomycin and cotrimoxazol, being regarded as a multiresistant and potentially virulent isolate. Negative results (sensitive) for levofloxacin, tetracyclin and cotrimoxazol demonstrated a variation of the antimicrobial profile for another Streptococaceae isolate, Gemella haemolisans, collected from Hospital C in M. pharaonis; meanwhile the results for another G. haemolisans isolate from Hospital A, showed resistance to penicillin pneumococci and erythromycin. However, a G. morbilorum isolate from Hospital B, isolated from T. melanocephalum, demonstrated resistance towards penicillin pneumococci, penicillin streptococci, amoxicillin pneumococci and vancomycin. Other Gram-positive bacteria, such as Baccilus spp. were classified only on a morphological basis. Tables 2 and 3 present bacteria species and their respective antimicrobial profiles.

With respect to the three *Enterococcus faecalis* strains tested, two were resistant to penicillin, amoxycillin, and ampicillin and sensitive to erytromicin, sulphazotrin, gentamicin, cephoxytine, vancomycin, clyndamicina, cephalotin, oxacillin and tetracycline and the other strain did not present a significant resistance profile towards the same drugs tested by the disk diffusion method. Of the two *E. faecium* strains tested, one presented an intermediary profile towards amoxycillin and amoxicillin, but was sensitive to all the other antibiotics tested.

Discussion

These species are common domestic ants and rather difficult to control (Campos-Farinha *et al.* 1997, Bueno & Campos-Farinha 1999 a,b) and have also been collected in other studies conducted in Brazilian hospitals. Whilst Fowler *et al.* (1993) and Peçanha (2000) detected bacteria associated with these species others have not (Bueno & Fowler 1994, Santos *et al.* 2001, Zarzuela *et al.* 2002).

The diversity of ant species found in hospitals is high, as mentioned by Peçanha (2000), which found seven species of ants in two public hospitals in the city of Sorocaba, SP. The authors classified 42% of samples as T. melanocephalum, P. longicornis 24.5%, M. pharaonis 17%, Brachymyrmex spp. 8%, Camponotus spp. 5%, Monomorium floricola (Jerdon) 3%, and 0.5% *Crematogaster*. Besides those aforementioned, Fowler *et al*. (1993) found six more species of ants, Crematogaster sp., Solenopsis sp., Conomyrma sp., Wasmannia auropunctata, Pheidole spp. and Linepithema. They described ants associated with the following bacteria: S. aureus, Enterobacter, E. faecalis, S. rubidae, Acinetobacter, Klebsiella and Serratia marcescens. Our results found at least three of these microorganisms associated with ants. Delabie et al. (1995) found a vast array of ant species in hospital environment in the city of Ilheus, BA, with 36% classified as T. melanocephalum, P. longicornis in 30% of samples, P. megacephala in 21%, and Solenopsis globularis Forel in 5%. A 1989 survey of Bueno & Campos-Farinha (1999 a) at Hospital das Clínicas of Botucatu, SP, concluded that the infestation by ants reached 16% and T. melanocephalum was the protagonist among the species collected. Five years later in a second survey, the same authors described indices of infestation of 72.7% and the predominant species was P. longicornis. Whilst Santos (2001) collected 29 species of ants in two hospitals in the city of Viçosa, MG, among those species the authors found Anochetus diegensis Forel, Crematogaster gp.sp. quadriformis (Forel), Ectatomma edentatum (Roger), S. saevissima, T. melanocephalum, Mycocepurus smithi (Forel), Odontomachus haematodus (L.), Tetramorium bicarinatum (Nylander), T. simillimum, two species of Brachymyrmex, six species of Camponotus, two species of Monomorium, and 10 species of Pheidole.

Gram-negative bacteria are responsible for a large number of nosocomial infections, particularly in patients confined for long periods and treated with an intravenous drip (Gayvallet-Montredon et al. 2002). Some of the microorganisms detected in ants have been linked to potentially serious problems in hospitals. Acinetibacter baumanii normally grows on human skin (Madigan et al. 2000), but can cause respiratory and post-surgery infections. This bacterium is frequently described as an antibiotic-resistant hospital pathogen (Lopez-Otsoa et al. 2002, Cisneros & Rodriguez-Bano 2002), including in Brazilian hospitals (Gales et al. 1986, 2003; Levin et al. 1999). Enterobacter can pose a significant threat to children that have contracted diarrhea in hospitals (Peçanha 2000), and E. cloacae can be particularly serious for neonates in

Table 1. Bacteria found in three species of ants (*T. melanocephalum*, *P. longicornis* and *M. pharaonis*) collected from Intensive Care Units (ICU) and Infirmaries (INF) in three hospitals (A, B and C) in Campos dos Goytacazes, RJ, Brazil.

Ant species	Bacterium species	Hospital/collecting site
T. melanocephalum	Bacillus spp.	A(3), C(2,4,8)
	Enterobacter amnigenus	A(5), C(9)
	Enterobacter cloacae	A(3), B(6,7), C(2)
	Enterococcus faecalis	A(2,3)
	Klebsiella pneumoniae pneumoniae	A(2,3),B(6,7),C(2)
	Staphylococcus saprophyticus	A(2)
	Acinetobacter baumanni	B(4), C(4)
	Gemella morbillorum	B(6)
	Staphylococcus epidermidis	B(4)
	Staphylococcus equorum	B(6)
	Klebsiella oxytoca	C(4)
	Staphylococcus aureus	C(8)
	Staphylococcus simulans	C(4)
	Staphylococcus warneri	C(8)
P. longicornis	K. pneumoniae pneumoniae	B(1)
	Serratia rubidae	B(1,7)
	Staphylococcus cohnii	B(7)
	Bacillus spp.	C(2,8)
	Enterobacter agglomerans	C(4)
	Enterobacter cloacae	C(8)
	Gemella haemolysans	C(4)
	Gemella morbillorum	C(4)
	Staphylococcus aureus	C(4)
M. pharaonis	Enterobacter agglomerans	A(5)
	Enterococcus faecalis	A(3)
	Enterococcus faecium	A(5)
	Gemella haemolysans	A(3)
	K. pneumoniae pneumoniae	A(3,5)
	Streptococcus acidominimus	A(5)
	Staphylococcus lugdunensis	A(5)

Collection sites: 1 - General ICU, 2 - pediatric ICU, 3 - general INF, 4 - pediatric INF, 5 - infectious and parasitic diseases INF, 6 - Post-surgery INF, 7 - gynecological INF, 8 - angiology INF, 9 - trauma INF

intensive care units (Dealler 2002). Klebsiella oxytoca and K. pneumoniae pneumoniae are considered highly pathogenic and frequently fatal (Cichon et al. 1997, Gebel et al. 2002), and antibiotic-resistant K. oxytoca has been responsible for infant deaths in hospitals (Reiss et al. 2000). Interestingly, in the present work the presence of resistant K. oxytoca was detected in a pediatric INF, which was the most visited area by ants. Strains of Staphylococcus

epidermidis resistant to methicillin were recently isolated from patients in Campos dos Goytacazes, RJ, and PCR analyses indicated the presence of the *mecA* gene (Vieirada-Motta *et al.* 2000a), which confers resistance to methicillin in staphylococci.

Clinical strains of *S. aureus* and *S. epidermidis* have been isolated before in pediatric INF hospitals of Campos dos Goytacazes, and were found to be multi-resistant,

Table 2. Staphylococcus spp. and their antibiotic resistance profile isolated in hospitals from Campos dos Goytacazes, RJ, Brazil.

Bacteria	N	A	В	С	D	Е	F	G	Н	I	J	K	L	M	О	P	Q	R
S. aureus ¹	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. aureus ²	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Staph ³	7	7	6	6	4	2	6	5	3	1	2	2	4	0	0	2	0	2

¹Collected from *P. longicornis* in hospital C.

A - penicillin, B - oxacillin, C - coagulase(-)oxacillin, D - cotrimoxazol, E - gentamycin, F - erythromycin, G - clindamycin, H - tetracyclin, I - minocylin, J - vancomycin, K - teicoplanin, L - rifampicin, M - norfloxacin, O - levofloxacin, P - fusidic acid, Q - nitrofurantoin, R - quinupristin-dalfopristin

according to the disk diffusion method, agar dilution and PCR analysis (Vieira-da-Motta *et al.* 2000a, 2001a). Strains of *S. aureus* of bovine origin from Campos dos Goytacazes region displayed resistance to several antibacterial drugs and high genetic variability, which was indicated by high polymorphism, and these characteristics were attributed to the intense traffic of dairy cattle in the region (Vieira-da-Motta *et al.*, 2001a). Furthermore, no MRSA (methicillin resistant *S. aureus*) were found among bovine isolates (Vieira-da-Motta *et al.* 2000b).

Since Campos dos Goytacazes is the biggest city in the Northern area of Rio de Janeiro State and patients come from far away seeking medical assistance, one can speculate that different isolates of *S. aureus* and coagulase-negative *Staphylococci* (CoNS) isolated from ants that colonize hospitals in the municipality could play a similar epidemiologic role provided by bovine isolates, assuming a potential health risk to hospital communities. Moreover, the fact that methicillin resistance is attributed to acquisition of the *mecA* gene from a CoNS *S. sciuri* (Wu *et al.* 1996) and the presence of CoNS isolates (i.e. *S. haemolyticus* e *S. epidermidis*) in hospitals visited by ants presented in this

study, is a matter for preoccupation in such environments, since vancomycin-resistance of the same species have been previously identified in other studies (Veach *et al.* 1990, Herwaldt *et al.* 1991). The results from two CoNS *S. saprophyticus* and *S. equorum*, each vancomycin-resistant, highlight their importance and could be seen as a risk factor for the health institutions studied.

S. epidermidis is among the microorganisms able to form biofilms in hospitalar material and responsible for nosocomial infections (Dunne et al. 1993). One strain of S. epidermidis was isolated from Tapinoma melanocephalum from pediatric INF in hospital B, which may represent a health risk. It has been estimated that 65% of nosocomial infections are associated with biofilms, which causes annual losses of approximately one billion dollars in hospital stays and treatment (Costerton et al. 1995, Archibald & Gaynes, 1997, Potera, 1999).

In Brazil, in Rio de Janeiro State and in other Brazilian states, MRSA have been isolated from hospital institutions and from other risk environments, with a clear participation in nosocomial and invasive infections in AIDS patients and as a potential reservoir for food poisoning (Teixeira *et al.*)

Table 3. Gram-negative bacteria resistant to antibiotics in hospitals in Campos dos Goytacazes, RJ, Brazil. N is the number of isolates tested.

Bacteria	N	A	В	С	D	Е	F	G	Н	I	J	K	L	M	О	P	Q
A. baumannii	2	2	1	1	1	1	2	1	0	0	0	2	2	0	1	1	0
E.agglomerans	3	2	0	0	2	0	3	3	0	0	0	0	1	0	1	0	0
E. amnigenus	2	2	2	2	2	1	2	2	0	0	0	1	1	2	1	1	1
E. cloacae	4	4	4	3	3	2	4	4	1	1	0	3	1	2	2	2	2
K. oxytoca	1	1	0	1	1	1	1	0	0	0	1	1	0	1	1	0	1
K.pneumoniae. pneumoniae	12	12	0	2	10	1	4	1	0	0	1	2	1	1	2	2	2
S. rubidae	6	3	0	0	3	0	5	2	0	0	0	5	1	0	0	0	0

^{**}The antibiotics piperacillin+tazobactam, meropenem, imipenem, amikacin and ciprofloxacin were also screened, but there were no positive results.

²Collected from *T. melanocephalum* in hospital B.

³Coagulase-negative Staphylococci

A - amoxicillin, B - amoxicillin-clav.acid, C - piperacillin, D - ticarcillin, E - ticarcillin-clav.acid, F - cephalothin, G - cefoxitin, H - cefotaxim, I - ceftazidim, J - cefepim, K - cefuroxim, L - ceftazidime 1, M - cotrimoxazol, O - tobramycin, P - gentamicin, Q- netilmicin

1996, Resende & Figueiredo 1997, Soares *et al.* 1997, Santos *et al.* 1999).

In addition to the antibiotic resistance profile in staphylococci (Lowy 1998), food poisoning also represents a threat to patients, mainly in the hospital environment (Tortora et al. 1996), because proteins secreted by the organisms display superantigenic characteristics as they promote massive lymphocyte stimulation leading to toxic shock syndrome and food poisoning symptoms (Bergdoll 1990, Torres et al. 2001). The occurrence of S. aureus is of particular interest, since many foods visited by ants provide ideal sites for bacterial multiplication, offering a serious risk of hospital food contamination with enterotoxin production. Toxigenic S. aureus isolates in milk from bovine producing staphylococcal enterotoxins have been previously isolated in Rio de Janeiro State (Vieira-da-Motta et al. 2001b,c). Moreover, testing for detection of toxin genes (enterotoxin, exfoliatin, and TSST-1) and methicillin resistance of two S. aureus, as well as the CNS isolates from this work are under investigation.

The finding of *E. faecalis* and *E. faecium* with phenotypic sensibility to antimicrobial tested in this work do not implicate in the absence of risk to patients in the hospitals visited by ants. These bacteria are responsible for *S. aureus* resistance to vancomycin throughout gene transfer (Morris *et al.* 1995). Thus, a future molecular investigation should eliminate the potential risk of antimicrobial resistance gene(s) carrier by the strains isolated in the present work.

These results emphasize the importance of considering ants, especially *T. melanocephalum* which was present in most studies, as potential vectors of diseases caused by virulent and emerging resistant bacteria. Sanitation measures, monitoring numbers of ants and keeping them under control in hospital environments is advised.

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