Original Article

Ant-associated bacteria collected from hospitals in Anápolis – GO

Bactérias associadas a formigas coletadas em hospitais em Anápolis – GO

Bacterias asociadas a hormigas recolectadas de hospitales en Anápolis – GO

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Submitted: 08/06/2019

Accepted: 01/20/2020

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ABSTRACT

Background and Objectives: hospitals are prone environments for the establishment and spread of insects, especially ants. In addition to their adaptive capacity, ants live in mutualism with other living beings such as fungi and bacteria, which increases the risk of nosocomial infections. This study aimed to identify the bacterial microbiota associated with intrahospital ants in the city of Anápolis, Goiás, and to discuss the role of such agents in the development of nosocomial infections and consequent risk for hospitalized individuals. **Methods:** ant traps were set up in two hospitals in Anápolis to capture them in the ward sectors as well as the intensive or semi-intensive care units and the nutrition sectors. The traps were left for a predetermined period in the respective locations and were then taken to the UniEvangélica

Microbiology Laboratory for culture, sowing and bacterial identification. **Results:** three collections were performed in each of the hospital sectors of each hospital institution. The following microorganisms could be isolated: *Staphylococcus* spp., Gram-positive bacilli, *Klebsiella ozaenae*, *K. rhinoscleromatis*, *Escherichia coli* and *Yersinia pseudotuberculosis*. **Conclusion:** we can conclude that ants can act as vessels for microorganisms. This fact suggests that ants may favor infections in the hospitals. However, the relationship between ant population and incidence of infections in hospitals remains uncertain, and studies are necessary to associate these variables.

KEYWORDS: Hospital Infection. Disease Vectors. Infection Control. Insect Vectors. Public Health.

RESUMO

Justificativa e Objetivos: os hospitais são locais propícios para a instalação e propagação de insetos, especialmente formigas. Essas, além da sua capacidade adaptativa, vivem em mutualismo com outros animais, como fungos e bactérias, o que confere risco elevado para infecções nosocomiais. O presente estudo teve como objetivo identificar a microbiota bacteriana associada com formigas intra-hospitalares na cidade de Anápolis, Goiás, e discutir o papel de tais agentes no desenvolvimento de infecções hospitalares e o consequente risco para indivíduos hospitalizados. **Métodos:** foram montadas armadilhas para formigas em dois hospitais da cidade de Anápolis a fim de capturá-las nos setores de enfermaria, unidade de terapia intensiva/semi-intensiva e nutrição. As armadilhas eram deixadas por um período pré-determinado nos respectivos setores e depois eram levadas ao Laboratório de Microbiologia da UniEvangélica para cultivo, semeadura e identificação bacteriana. **Resultados:** foram realizadas três coletas em cada um dos setores de cada instituição hospitalar. Foi possível isolar os seguintes microrganismos: *Staphylococcus* spp., bacilos Gram-positivos, *Klebsiella ozaenae*, *K. rhinoscleromatis*, *Escherichia coli* e *Yersinia pseudotuberculosis*. **Conclusão:** pode-se concluir que as formigas podem atuar como veículos para microrganismos. Esse fato sugere que podem favorecer o processo de infecção em usuários de assistência hospitalar. Entretanto, permanece incerto a relação entre população de formigas e incidência de infecções nos hospitais, sendo necessário realizar estudos para associar tais variáveis.

DESCRITORES: Infecção Hospitalar. Vetores de Doenças. Controle de Infecções. Insetos Vetores. Saúde Pública.

RESUMEN

Justificación y Objetivos: los hospitales son ambientes propicios para la instalación y propagación de insectos, especialmente hormigas. Además de su capacidad de adaptación, estos animales viven en mutualismo con otros, como los hongos y las bacterias, lo que confiere un alto riesgo de infecciones nosocomiales en los humanos. El presente estudio tuvo como objetivo identificar la microbiota bacteriana asociada con hormigas intrahospitalarias en la ciudad de Anápolis, Goiás, y analizar el papel de dichos agentes en el desarrollo de infecciones hospitalarias y el riesgo para las personas hospitalizadas. **Métodos:** se instalaron trampas para hormigas en dos hospitales de la ciudad de Anápolis para capturarlas en los sectores de

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enfermería, unidades de cuidados intensivos, de cuidados semiintensivos y nutrición. Las trampas se dejaron durante un período predeterminado en los sectores respectivos y después se las llevaron al Laboratorio de Microbiología UniEvangélica para su cultivo, siembra e identificación bacteriana. **Resultados:** se realizaron tres colectas en cada uno de los sectores hospitalarios de cada institución hospitalaria. Se pudieron aislar los siguientes microorganismos: *Staphylococcus* spp., bacilos Gram positivos, *Klebsiella ozaenae*, *K. rhinoscleromatis*, *Escherichia coli* y *Yersinia pseudotuberculosis*. **Conclusiones:** se concluye que las hormigas pueden servir como vehículos de microorganismos. Esto sugiere que pueden favorecer el proceso de infección a los usuarios de atención hospitalaria. Sin embargo, la relación entre la población de hormigas y la incidencia de infecciones en los hospitales sigue siendo incierta, y se necesitan más estudios para asociar estas variables.

PALABRAS CLAVE: Infección Hospitalaria. Vectores de Enfermedades. Control de Infecciones. Insectos Vectores. Salud Pública.

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INTRODUCTION

Urbanization, when associated with poor forest conservation, can create fundamental conditions for various infectious diseases. Several animals such as fleas, lice, and rats (vectors for bubonic plague, rickettsiosis and leptospirosis, respectively) already have a consolidated degree of attention and epidemiological importance. Ants, however, are underestimated in their ability to contribute directly to health hazards, and may be of comparable importance to the abovementioned vectors. Frequently seen as more of a nuisance, there is the potential for ants to act as vessels of pathogenic microorganisms, highlighting this role within hospital environments. In recent years, however, concern about ants as mechanical vectors has also extended to household environments.[1,2]

As their potential for harm is underestimated, safety measures intended to reduce this population inside medical institutions are neglected, even if sometimes present. If its relevance was adequately stated, sanitary, hygienic and structural measures could have been implemented to efficiently and effectively reduce ant circulation and possibly have a positive impact on health indices. In developed countries, pest control in hospitals is already of paramount importance.[3,4]

Architectural features (such as gaps and breaches on the floor, sockets and electrical circuits, as well as its related structures like cabinets, machines and appliances), proximity to households and continuous flux of people are some of the main factors that make up for the high ant population in nosocomial environments. Since ants have mutualistic relationships with

other living beings, such as fungi and bacteria, it is to be expected that the flow of these microorganisms is also related to the ant population.[5]

The harmful presence of ants in a nosocomial environment is emphasized in view of the health problems of patients, especially hospitalized patients, and health professionals due to the risks of spread of pathogens and subsequent cross-infection. This condition can be observed as a risk factor for increased exposure to various types of etiological agents and consequently to infection, as well as the extensive treatment of broad-spectrum antibiotics, which accentuates the emergence of bacteria with antimicrobial resistance.[6]

In Brazil, it is estimated that 5-15% of hospitalized patients will develop an hospital infection. Among such infections are bacterial pneumonia and infections in the genitourinary tract, in the bloodstream and in surgical wounds, as well as sepsis; these conditions are the majority of diseases caused by bacteria in the nosocomial environments and are often subject to poor prognosis. Pathogens capable of causing such damage commonly live in symbiosis with ants.[7-9]

Various bacteria, with emphasis on the genera *Staphylococcus*, *Streptococcus*, *Serratia*, *Klebsiella*, *Acinetobacter*, *Enterobacter*, *Salmonella*, *Escherichia* and *Enterococcus*, have been described to be associated with ants in hospital settings. *Pseudomonas* spp., *Hafnia alvei*, *Enterobacter aerogenes*, *Burkholderia cepacia* were also found, albeit less frequently.[2,3,5,9-13]

In this scenario, this study aimed to verify the bacterial microbiota associated with ants collected in two hospitals in the city of Anápolis, Goiás. We also sought to relate this fauna with the possible health risks of patients with regard to hospital infection.

METHODS

This was a descriptive, cross-sectional study, conducted from December 2018 to June 2019 in two hospitals in the city of Anápolis (referred to as hospitals A and B; the former was part of the public network while the latter was private) ; in the following sectors: wards, intensive care unit/semi-intensive care unit (ICU and S-ICU) and nutrition service (kitchen). Nine collections were performed in each of the hospitals, covering all the sectors mentioned.

To carry out the study, it was not necessary to issue an opinion of the Ethics and Research Committee, since the methodology does not fit the specifications for this.

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Sample collection

The material preparation phase consisted of autoclave sterilization in the Microbiology Laboratory of UniEvangélica (Labbas) of the materials that made up the ant traps: 1 mL of bee honey in test tubes. After this process, the materials were taken to hospitals, properly packed, and then allocated in the respective sectors. Each trap was assembled as follows: for the experimental tubes, two open test tubes containing honey as bait were positioned on the ground to attract ants. For the control tube, an open test tube was supported in a beaker, contained within a water-filled Petri dish that prevented ant entry in order to control for air contamination. Traps were left for three hours in the afternoon for one-third of the collections and for 12 hours in the night for the remaining two-thirds.

Sample culture

After collection, the tubes were closed and taken to Labbas. All tubes, regardless of ant presence, were filled with brain heart infusion (previously sterilized in autoclave and incubated in an oven at 35ºC for 24-48 hours. After this process, all BHI tubes that presented turbidity (thus indicating growth) were sowed in plates containing Mannitol salt agar, MacConkey agar and nutrient agar, all previously sterilized. The plates were subsequently incubated at 37 ºC for 24-48 hours.

Identification of bacterial isolates

The bacterial identification phase was performed according to plate growth characteristics and staining aspects related to Gram staining. Gram-positive bacteria were submitted to catalase testing. Gram-negative bacteria were submitted to oxidase testing and subsequently to the Bactray systems I and II (oxidase-negative bacteria) and Bactray III (oxidase-positive bacteria). The Schaeffer-Fulton staining method was also used to identify endospores in Gram-positive bacilli. These data were correlated with the morphological aspects of bacterial colonies under optical microscopy.

RESULTS

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Three collections were performed in each hospital for each hospital sector, totaling 18 collections during the study period. A total of 54 study tubes were collected, of which 36 were experimental (18 in hospital A and 18 in hospital B) and 18 were control (9 in hospital A and 9 in hospital B). Each study sector of the hospital received 12 experimental tubes (6 in hospital A and 6 in hospital B). Frequency of experimental tubes whose traps were successful in ant capture, as well as their respective place of collection, are shown on Table 1.

Table 1. Absolute frequency of experimental tubes with trapped ants from different sites of two hospitals (A and B) in the municipality of Anápolis, GO.

Hospital	Sector	AF	Ant
A	S-ICU	4	*Paratrechina longicornis*
	WAR	-	-
	NUT	2	*Paratrechina longicornis* *Tapinoma melanocephalum*
B	ICU	1	*Paratrechina longicornis*
	WAR	1	*Tapinoma melanocephalum*
	NUT	4	*Paratrechina longicornis* *Tapinoma melanocephalum*

AF: absolute frequency; WAR: wards; NUT: nutrition service

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Of the total of 36 experimental tubes, 33% succeeded in capturing ants (n = 12). In hospital A, 66% (n = 4) of the experimental tubes with ants came from the S-UTI and 34% (n = 2) from the nutrition service. In hospital B, 66% (n = 4) of the experimental tubes with ants came from the nutrition sector, 17% (n = 1) from the ICU and 17% (n = 1) from the wards sector. The ward sector of hospital A was the only one in which it was not possible to capture ants. There were two captured species: *Paratrechina longicornis* and *Tapinoma melanocephalum*. Bacterial colony growth was identified in the trap experimental tubes, regardless of ant presence. It was inferred that in the positive experimental tubes without ant capture there might have been contact of ants with the bait temporarily, once the strains isolated

in the control tubes of the respective traps were excluded. In this scenario, Chart 1 demonstrates the bacteria identified in the experimental tubes.

Chart 1. Bacteria isolated in experimental tubes (regardless of ant presence) from traps of different locations in two hospitals (A and B) in the municipality of Anápolis, GO.

Hospital	Sector	Bacteria
A	S-ICU	*Staphylococcus* spp.* *Klebsiella ozaenae**
	WAR	*Staphylococcus* spp. Gram-positive bacilli *Yersinia pseudotuberculosis*
	NUT	*Staphylococcus* spp. Gram-positive bacilli
B	ICU	*Staphylococcus* spp.
	WAR	*Staphylococcus* spp.* *Escherichia coli**
	NUT	*Staphylococcus* spp.* *Klebsiella rhinoscleromatis** *Escherichia coli* Gram-positive bacilli

AF: absolute frequency; WAR: wards; NUT: nutrition service

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The isolation of *Staphylococcus* spp. was the most frequent, found in all experimental tubes positive for bacteria. Gram-positive bacilli were identified in the wards (hospital A) and nutrition (hospital A and B) sectors, although not directly associated with trapped ants. The growth of *E. coli* strains in the wards and nutrition sectors of hospital B was verified, associated with ants collected in the first sector mentioned. Furthermore, there was growth of microorganisms of the genus *Klebsiella*, identifying *K. ozaenae* in the ICU sector of hospital A and *K. rhinoscleromatis* in the nutrition sector of hospital B, both in association with ant samples from the respective sites. *Yersinia pseudotuberculosis* was identified in the ward sector of hospital A.

In one of the tubes with positivity for Gram-positive bacilli, the formation of endospores was also identified. It was not possible to identify them precisely, but morphological similarity (microscopic and macroscopic) was observed with *Bacillus subtilis*.

DISCUSSION

Similarly to the 2010 study,[14] the most commonly found bacterial genus was *Staphylococcus*. In addition to their great adaptability and ability to cause infections, staphylococci are large representatives of hospital infections in the current context. Although it is associated with pathological entities of benign course, *S. aureus* is capable of generating severe and invasive infections, possibly even fatal, specially in patients debilitated due to hospital infection, with; the skin, soft parts and lungs are the main sites of relevance.[14,15]

In a hospital in the city of Sumaré, São Paulo, *S. aureus* was the microorganism most associated with deaths from hospital infection, mainly associated with pneumonia and bloodstream infection.[16] In reinforcement of these results, a study conducted in Minas Gerais identified that all strains of *S. aureus* associated with ants collected in the ICU and operating room were resistant to oxacillin, configuring them as bacteria capable of causing infection with a worse prognosis.[17]

Coagulase-negative staphylococci (CNS) comprise a broad group of microorganisms, which are mainly represented by pathogenic species *S. epidermidis*, *S. saprophyticus* and, less commonly, *S. haemolyticus*. Unlike *S. aureus*, of universal distribution, these pathogens are preferably associated with the hospital environment and have the ability to infect bloodstream

and urinary system, mainly. Although such agents are classically associated with colony formation in vascular catheters and prostheses primarily, their association with ants is not irrelevant, since it is known that some of these arthropods are attracted to clean and even sterile materials.[18]

In the study on Gram-positive bacteria associated with ants conducted in a hospital in Rondônia, *S. epidermidis* and *S. saprophyticus* represented half of the microorganisms found. In addition, they presented resistance to vancomycin and oxacillin. Strains of *S. aureus* comprised 13% of the results and showed resistance to oxacillin. CNS was the second group of microorganisms in association with ants most prevalent in a hospital in Botucatu, São Paulo, making up 20% of bacteria found.[9.19]

The genus *Klebsiella* is of great importance in the context of hospital infection, especially *K. pneumoniae*, a bacterium associated with severe opportunistic infections in hospitalized patients. Although this species was not isolated in the present study, other representatives of the genus also have the potential to cause disease. There are reports of otitis media, mastoiditis, bacteremia, soft tissue infection and urinary tract caused by *K. ozaenae*, an ant-associated bacterium also found in a hospital in the state of São Paulo. The role of *K. rhinoscleromatis* in the development of severe nosocomial infections is uncertain, but it does not seem to be as relevant. It is known, on the other hand, about the relevance of this agent in the pathogenesis of rhinocleroma.[10,20,21]

Gram-positive bacilli were found in several ant samples in the in-hospital context in other studies of similar methodologies. This bacterial morphology encompasses a considerable number of species; in the clinical context, the most relevant are *B. anthracis, B. cereus and Listeria monocytogenes*. *L. monocytogenes* and *B. cereus* present gastroenteritis-related pathogenesis, while *B. anthracis* has a variable, aggressive and generally systemic clinical spectrum. Since B. cereus and B. anthracis are spores-forming bacteria, they easily adapt and propagate through the environment, a virulence factor that confers a certain independence of the transmission through mechanical vectors, although it does not cancel this importance. *B. subtilis* is a bacterium harmless to the human species and with probiotic value.[3,9,11,22,23]

E. coli strains have been reported in studies in the states of Bahia and São Paulo, although not associated with food preparation sectors as in this study. This bacterium holds different commensal and pathogenic strains, and among those that caused diseases the Shiga-toxin producing (STEC) and the enteropathogenic pathotypes stood out. The main disorders

caused by such microorganisms involve gastrointestinal symptoms; however the clinical profile may present greater severity depending on the strain. Hemolytic uremic syndrome, hemorrhagic colitis and even acute renal failure are consequences of infection by the enterohemorrhagic*E. coli* strain (EHEC) – a pathotype of STEC. The strain *E. coli* O157:H7 is the best known ECEH serotype and has epidemiological importance due to its potential for gastrointestinal outbreaks in several countries. Its tropism by the gastrointestinal system reinforces the danger of its association hospital kitchen ants.[2,10,24]

The enterobacterium *Y. pseudotuberculosis* found in this study was not described in other similar studies, but it was possible to isolate an organism of the same genus (*Y. pestis*) in a hospital in the city of Taubaté, São Paulo. The *Y. pseudotuberculosis* is transmitted via fecal-oral route either by contaminated water or food or by direct contact with infected individuals. The clinical spectrum includes fever, abdominal pain, diarrhea, and extra-abdominal symptoms such as reactive arthritis and erythema nodosum.[11,25]

As such, we can conclude that ants in hospital environments in the city of Anápolis also carry pathogenic and non-pathogenic bacteria, capable of causing various types of infections and, therefore, should be seen as important mechanical vectors of microorganisms by hospital infection control committees. However, the relationship between the presence and quantity of ants and the occurrence of hospital infections is still debatable. As such, additional studies should be developed in order to associate these variables.

It was possible to reproduce results found in other studies of the same theme, reinforcing the previously known data. On the other hand, the non-reproducibility of otherwise common bacterial isolations, such as of the *Streptococcus* spp. and *Enterococcus* spp., may be the result of the environmental variables characteristic of the research field, as well as slight methodological differences. This study contributes to the national characterization of the microbiota associated with ants, especially in the state of Goiás, given the scarcity of studies on the field in the state.

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ACKNOWLEDGMENTS

The authors thank the Centro Universitário de Anápolis - UniEvangélica for funding the study through the Scientific Initiation Program and for the full availability of Labbas during the research period. They also thank all Labbas technicians, particularly Hallyson Kim Lee Sato,

for the great assistance offered during the stages carried out on site. Furthermore, they thank the hospital infection control committees of the participating hospitals for allowing and facilitating the process of setting up traps and collecting ants.

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Gabriel Garcia Cunha Lopes, Geraldo Porto Magalhães Netto, Larissa Amorim Silva, Leandro Norberto da Silva Júnior and Rodrigo Scaliante de Moura contributed to the conception, design, analysis and writing of the article;

Gabriel Garcia Cunha Lopes, Geraldo Porto Magalhães Netto, Larissa Amorim Silva, Leandro Norberto da Silva Júnior and Rodrigo Scaliante de Moura contributed to the planning and design of the article, review and final approval;

All authors approved the final version of the manuscript and declared themselves responsible for all aspects of the work, guaranteeing their accuracy and integrity.

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