SCIENTIFIC NOTE

A New Sterile Technique Effective on Capturing Tramp Ants for Microbiological Investigations

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Nova Técnica Estéril Eficiente para Captura de Formigas Andarilhas para Investigações Microbiológicas

RESUMO - Formigas andarilhas são organismos excepcionais, que têm papel definitivo e importante no transporte de patógenos. O objetivo deste estudo foi desenvolver uma técnica microbiologicamente estéril para coletar formigas de áreas contaminadas. As armadilhas compostas por uma placa de Petri contendo meio de cultura foram testadas para verificar a aplicabilidade do sistema. Foi observada correlação positiva entre o crescimento de microrganismos e a presença de formigas dentro ou ao redor das armadilhas. A técnica descrita demonstrou ser útil para coletar formigas de diferentes ambientes, além de contribuir na vigilância de microrganismos patogênicos que representam problema para a saúde pública.

PALAVRAS-CHAVE: Saúde pública, microrganismo patogênico, *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli*, *Enterobacter*

ABSTRACT - Tramp ants are an outstanding group of organisms that have a definitive and important role in carrying pathogens. The purpose of this study was to develop a microbiological sterile technique of collecting ants from contaminated areas. The traps were composed of Petri dishes containing culture media, and were tested to verify the applicability of the system. A positive correlation between the microorganism growth and the presence of ants inside or around traps was observed. The technique described demonstrated to be useful to collect ants from different environments, helping the surveillance of pathogenic microorganisms that are of public health concern.

KEY WORDS: Public health, pathogenic microorganism, *Staphylococcus aureus*, *Klebsiella*, *Escherichia coli*, *Enterobacter*

Urban pests have followed mankind probably since the primitive humans began to store their crop surplus, providing a permanent source of food for opportunistic organisms. The human-inhabited environments are densely occupied areas with infinite supplies of food, permanently available to all urban organisms, especially tramp ants (Robinson 1996, McIntyre *et al* 2001).

Tramp ants are an outstanding group of organisms that have a defined and important role in carrying pathogens, as several pathogens were associated with ant infestations (Beatson 1972, Chadee & Le Maitre 1990, Rodovalho *et al* 2007).

Studies reporting the importance of ants as mechanical vectors of pathogenic microorganisms worldwide (Beatson 1972, Ipinza-Regla *et al* 1984, Chadee & Le Maitre 1990) and in Brazil (Fowler *et al* 1993, Peçanha 2000, Zarzuela *et al* 2005, Rodovalho *et al* 2007), have applied different sampling techniques, most often based on conventional methods of capturing ants. Despite the use of control samples, none of the techniques assured that the microorganisms collected were captured only and exclusively from the ants. The aim of this study was to investigate a microbiological sterile technique of collecting ants from contaminated areas to produce reliable data to ascertain that the microorganisms detected are in fact associated with the ant's body structures.

The principle of the method was based on the study published by Hughes *et al* (1989) using blood agar to attract ants. The trap consisted of a disposable Petri dish containing Mueller Hinton agar (Merck KGaA, Darmstadt, Germany) amended with 5% defibrinated sheep blood. The Petri dish was then sealed with semi solid paraffin (80% solid food grade paraffin, 20% liquid vaseline) to prevent the ants from moving from one plate to another inside the incubator after exposition (Fig 1A). Just before the exposition of the trap, a small hole not exceeding 2 mm was made at the side of the Petri dish with the help of a sterile hot needle, just above the

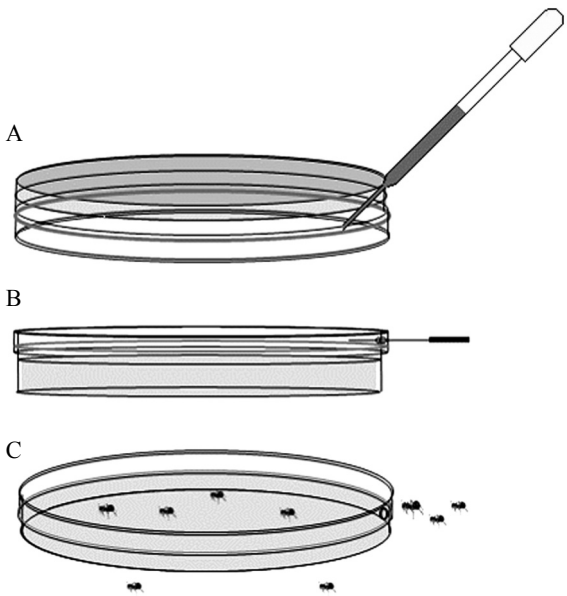

Fig 1 Schematic demonstration of a trap preparation to collect ants from the environment for microbiological purposes. A – Sealing traps with paraffin; B - Making a hole to let ants to enter the trap; C - Schematic observation of ants inside or around traps.

agar lane, big enough to let a small ant to fit in (Fig 1B). The hole was sealed with parafilm in a sterile environment, and maintained closed until exposition at the sampling site.

To test the system, traps were prepared accordingly and distributed in different establishments. A total of 120 Petri dishes or "traps" were distributed in 12 industrial kitchens (10 traps each), and the occurrence of ants inside and around each trap, the growth of microorganisms after incubation at the specific temperature, and the ant genera were observed and annotated.

At the study area, Petri dishes were positioned and the hole opened to permit the ants to get inside the trap (Fig 1C), and maintained exposed for 2h to 3h. Subsequently, the holes were sealed once again with parafilm, and the traps, containing or not ants, were taken to the laboratory and incubated for 24h at 35°C for microorganism isolation.

After the incubation period, plates were placed at 4°C for 15 min to immobilize the ants. The visible growth on the surface of the culture media was washed with 1 ml to 2 ml of sterile saline and recovered with the help of a sterile Pasteur pipette, and subjected to isolation on selective culture media for each organism of interest, by using the following media: MacConkey agar, Bismuth sulfite agar and Baird Parker agar (Merck KGaA, Darmstadt, Germany) supplemented with egg yolk tellutite emulsion (Newprov, Paraná, Brazil).

The ants from each Petri dish were collected with the help of a microbiological loop and transferred to vials containing 70% ethanol for further taxonomic study. The identification of cultured microorganisms was carried out according to Koneman *et al* (1997), and the identification keys organized by Bolton (1994) were used to identify ants to the genus level.

Scanning electron microscopy was conducted with ants collected from growth Petri dish and prepared according to Sallum *et al* (2002) in order to observe adhered bacteria to the ants' body structures.

Statistical treatment of the data was performed by using SPSS for Windows 8.0 software for frequency and Pearsons' linear correlation analysis. Significance was determined at the $P \leq 0.01$ level.

The composition of the culture media, blood agar, demonstrated to be effective to attract ants (Table 1). Besides its nutritive properties, the culture media resembles the surface of wounds in the healing process and post operatory incisions, which may be a huge attractive to ants mainly in hospitals (Hughes *et al* 1989).

The majority of the work available has used food as baits to attract ants, which can be an appropriate tool if the sole interest is to identify the ant infestation. The problem of such methods is that it is very difficult to avoid contamination originated from the surface or even from the air deposition when working with a microbiological process expected to be sterile.

In the method applied herein, the introduction of the opening to allow the ants to enter themselves the Petri dish and the fact that the plates were sealed, minimized the possibility of an accidental opening during the capture process, which differs from the original work of Huges *et al* 1989 (Fig 1A).

Table 1 Distribution of ants inside and around traps, visible growth of microorganisms after incubation, in each sampled area.

Exposition site	Ants inside traps* (%)	Ants around traps* (%)	Visible growth of microorganisms** (%)
A	0	0	NVGD
B	40	40	60
C	0	0	NVGD
D	50	60	50
E	20	40	40
F	30	40	50
G	0	10	NVGD
H	50	60	60
I	30	40	50
J	0	0	NVGD
K	50	50	100
L	60	60	80
Total	66.7	75	66.7

NVGD – No visible bacterial growth detected on the surface of culture medium inside the trap after incubation at 37°C for 24h; *Presence of ants inside or around the Petri dishes (traps) right before transfer traps to the laboratory considering 10 traps in each exposition site; **Visible growth of microorganisms colonies on the surface of the culture medium considered after incubation of Petri dishes (traps) at 37°C for 24h.

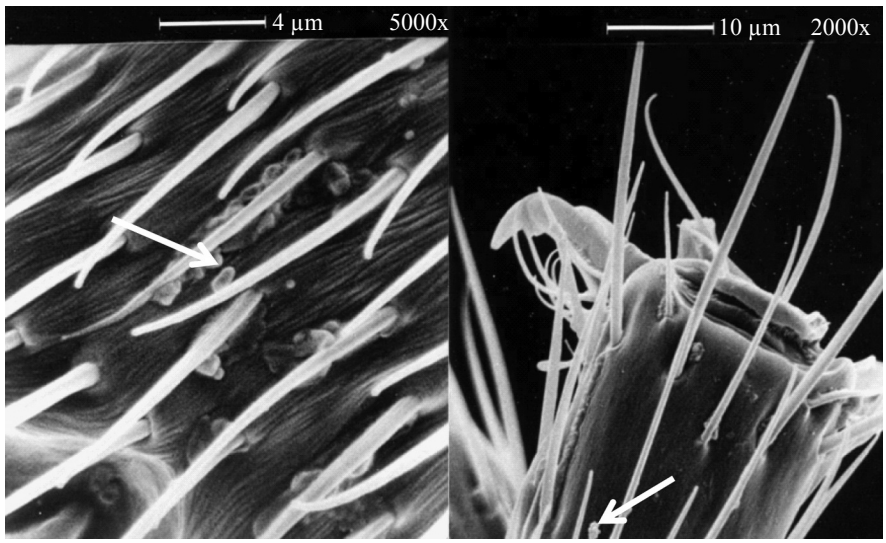

Fig 2 Scanning electron microscopy of a tramp ant demonstrating bacteria adhered to the body surface. Arrows demonstrate the position of bacterial cells.

The ant species observed inside or around the traps belonged to *Tapinoma*, *Solenopsis*, *Paratrechina*, *Pheidole* and *Monomorium* (data not shown). A positive correlation between the presence of ants around the traps and the growth of microorganisms on the surface of the culture medium was observed ($P \leq 0.01$). About 75% of the samples showed some kind of bacterial growth. These results demonstrate the vector or mechanical role of ants for the transport of pathogens.

The samples that demonstrated no bacterial growth also did not show the presence of ants either inside or around the plates. This corresponds to 25% of the total samples, which suggests that the method is secure against environmental, collector or other organism contamination.

Scanning electron microscopy observations of tramp ants confirmed the presence of bacteria adhered to the structures of the ant leg and demonstrated that microorganisms can get attached to the appendices when they walk through a contaminated surface (Fig 2).

In conclusion, the technique described herein demonstrated to be useful to collect ants from different environments, to evaluate mechanical capabilities of ants as vectors in hospitals or food preparation areas, but mainly aiding on the surveillance of pathogenic microorganisms that are of public health concern.

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