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Original Article

Ants as carriers of pathogenic bacteria in the Benghazi Medical Center (BMC)

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ABSTRACT

Ants are a common insects in hospitals environments; they can spread opportunistic bacteria in the areas where they forage. Many studies have confirmed the presence of pathogenic bacteria in/on ants collected from hospital environments. With the propagation of pathological organisms in the environment, its wondered that most common ants could transfer pathogens in spite of their small size. In this study, pathogenic bacteria were isolated from the exterior parts of ants that were collected from eight different locations in the Benghazi Medical Center (BMC), and it was also determined which ant genera were prevalent in these environments. A total of 125 ant samples were attracted by using carbohydrate traps. Each sample consisted of a group of five ants and put into test tubes containing brain and heart infusion (BHI) broth for the cultivation of fastidious pathogenic microorganisms . After 24 hours, sub-cultured on blood agar, Mannitol Salt Agar, and MacConkey Agar and incubated for 24 hours at 35/37°C. Clinical microbiological standards were followed for the Gram characterization, culture identification, and biochemical characterization. Subsequently, isolates were assessed for antibiotic-resistant. Isolated strains belonged to different species including: Staphylococcus aureus, Staphylococcus epidermidis, Corynebacterium spp (diphtheroides), Escherichia coli, and Enterobacter spp. The examination of isolated bacteria for antimicrobial susceptibility revealed that the most active antibiotic against these pathogens was Ciprofloxacin. Gram-positive bacteria showed resistance to Cefoxitin and Oxacillin while Gram-negative bacteria were resistant to Augmentin. It's also important to highlight that some pathogens show multi-drug resistance (MDR).

Key words: Hospital, ants, pathogenic bacteria, antibiotic resistance

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INTRODUCTION

Ants (*Formicidae*, Hymenoptera) live in symbiosis with humans and readily adapt to urban environments. Ants are typically omnivorous, and the majority of the animal proteins and fats in their diet come from insects and other arthropods that the foraging worker ants consume [1]. Ants are drawn also to foods and even medications, particularly those containing sugar, like other social arthropods [2]. Furthermore, these insects migrate to electronic devices in quest of thermal stability due to the architectural design, closeness to houses, weather interference, and temperature changes [3]. One of the primary habitats for ants is the hospital environment, and their presence can promote the spread of pathogenic microorganisms [4]. Although ants are possible disease carriers there is a limited knowledge about ants than there is about other insect pests such flies and cockroaches. A range of internal [5],[6], [5],[6], and external [1], [7], [8], bacterial species are unfortunately carried by ants and stick to their external surface, mainly their legs and mandibles. These appendages make contact with substrates like soil, pit latrines, and flooring inside, which the ants may use to acquire infections [1].

Many factors, including personal items carried by visitors to patients and sterilizing products, contribute to ant colonization in a hospital ward and may be responsible for

the movement of ants inside hospital buildings [9].

Many studies have indicated that several bacteria spp. have been isolated from ants including coagulase-positive staphylococcus, coagulase-negative staphylococcus, gram-negative bacilli [10] other Staphylococcus spp., Bacillus cereus [11] Proteus mirabilis and Pseudomonas spp. [12]. While a study about ants as carriers of antibiotic-resistant bacteria in hospitals has shown that some bacteria isolates, including those from the Acinetobacter, Streptococcus, Gemella, Klebsiella, and Enterobacter families, are multi-resistant [9]. Although not the primary cause of hospital illnesses, ants are nonetheless thought of as a possible transmission method. This exposure may alter the prognosis for immunosuppressed patients, who are vulnerable to developing infections, prolonging their hospital stay and raising the cost [3]. However, because little research has been carried out on this topic, there is currently a lack of knowledge regarding consumers' awareness, perceptions, and behaviors in relation to nuisance ants in hospitals and homes.

To our knowledge, this is the first study performed in a hospital in Libya so far concerning this research. The aims were to study the fauna of ants in the BMC as well as to identify the possible pathogens that ants carry and their patterns of resistance to antibiotics. Due to their enormous number and the easy accessible ways for them to enter the structure, they may cause harm, which is considered a threat and demands control.

MATERIALS AND METHODS

Ants collection

The study was conducted in April and June 2019 Ants were collected in 8 different environments in Benghazi Medical Centre, namely: i-Dialysis unit, ii-Operating Theater (OT), iii-Maternity and delivery ward, (iv antenatal ward, (v) Emergency room (ER), (vi) kitchen, cafeteria, (vii) reception, (viii) outdoors.

The method of capturing and microbiologically analyzing ants was adapted from Oliveira et.al [2]. The collection took place twice on the same day

at different sites, in the morning and in the early evening and a total of 25 plates containing carbohydrate baits (sugar and caramel sweets), nine baits attracted some ants, these plates were left in their place for one hour. Ants after that were carefully picked with sterilized tweezers and placed in the tube containing BHI broth. It is a liquid medium used for the cultivation of fastidious and non-fastidious microorganisms, including aerobic and anaerobic bacteria, from clinical specimens, food and environmental samples. Each tube contained five ants and was labeled with the original hospital environment . Throughout the collection process, the operator used sterile gloves. The tubes were then transferred to a box and transported to the laboratory of microbiology, where the microbiological analysis was conducted.

Microbiological Analyses

The ants were kept in BHI broth, and incubated at 35/37°C for 24 hours for bacteria enrichment and isolation. Next day, a loopful from the BHI broth were sub-cultured on blood agar, Mannitol Salt Agar, and MacConkey Agar, and incubated for 24 hours at 35/37°C. Negative cultures were re-incubated for 24 hours. The bacterial growth were identified by means of Gram staining techniques and a series of biochemical tests including indole, methyl red, Voges-Proskauer, and citrate (IMViC). Gram-positive cocci were characterized according to the cell morphology, and the catalase and coagulase tests.

Antimicrobial susceptibility

Antibiotic susceptibility test was done using the disk diffusion method on Mueller Hinton Agar, following the Clinical and Laboratory Standards. Ciprofloxacin, Levofloxacin, Cefoxitin, Cephalexin, Clindamycin, Erythromycin, Augmentin and, Oxacillin disks were used to evaluate resistance of the isolates of Gram positive bacteria. Ciprofloxacin, Aztreonam, Ceftazidime, Tazobactam/piperacillin, Imipenem, Cephalexin and , Augmentin were used to test E. coli and other gram negative bacteria.

Identification of ants

Ant species were identified with the help of an ecologist and entomologist professor, Terry McGlynn , is a professor of biology and director of undergraduate research at California State University- Dominguez

Hills and characterized by the use of an ant identification guide from Bayer Environmental Science [13].

RESULTS AND DISCUSSION

Of the 25 traps laid, 9 (36%) attracted ants, of which all of them were of the genus Monomorium and identified as Monomorium pharaonis (Pharaoh's Ant), (Table 1). In total, 125 individual ants were captured, with ants collected successfully from 8 different locations (88.8%) out of the initial 9 locations (Table 1). The isolation of microorganisms from the M. pharaonis samples revealed 28 bacteria containing six species, including four gram-positive bacteria, and two gram- negative bacteria table 1.

Table 1. Bacteria found on collected ants in multiple place in Benghazi Medical Center

Ants species	Bacterium species	Gram	Hospital/Collecting site
***M. pharaonis* (LINNAEUS, 1758),**	*S. aureus*	positive	B (1).
	Corynebacterium spp (diphtheroides)	positive	B (3) (4) (5) (6) (7) (8)
Common name:-	*S.epidermidis*	positive	B (3) (5) (6) (8)
Pharaoh's ant	*Micrococci*	positive	B (2)
	Enterobacter	negative	B (3)
	E. Coli	negative	B (8)

Places (B), collected from were: (1) Antenatal , (2) Cafeteria, (3) Kitchen, (4) Emergency room "ER", (5) kidney, (6) Gyne, (7) Outdoor, (8)Reception.

In contrast, a study conducted at the University of Bahia showed multiple ant species, including Pheidole spp., Tapinoma melanocephalum, Linepithema spp., and Crematogaster spp., which were collected from seven different locations around the hospital [2]. Additionally, in another study, ants were collected from different hospitals and different places and showed multiple species as well [3]. Although there are several types of ants that can be seen everywhere, in this study, only one species was detected in the BMC which is the M. pharaonis (Pharaoh ant) (table 1). The

Monomorium spp. have long been considered the most abundant house ant in the world. It is particularly notorious as a pest in hospitals, where it is known as a vector for disease [14]. An association between ants and bacteria it has been observed in various hospitals, raising concerns about the role of these insects as disease vectors [15]. The bacteria in this study were mostly gram-positive due to their availability in the environment. In particular, Corynebacterium spp (diphtheroides) was collected from most places in the hospital, followed by S.epidermis. Staphylococcus spp. (57.3%), were the most common bacteria isolated while the Enterobacter spp., and E. coli (gram negative) were the rarest, all having a 7% occurrence (Figure 1). Most of the ants hosted more than one bacterial species.

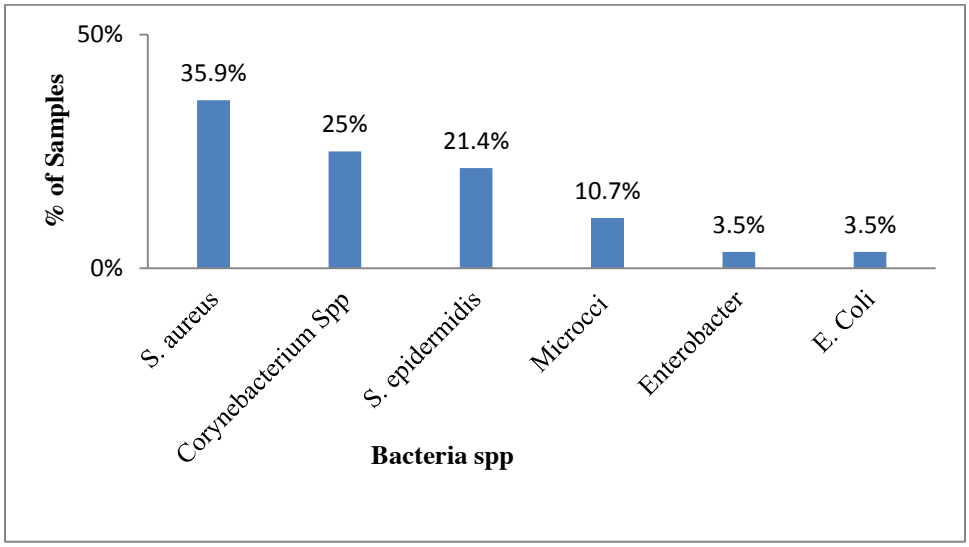

Figure 1. Percentage of bacteria spp. isolated from the captured ants

Corynebacterium spp are commonly observed in conjunction with nosocomial infections and the vast majority of them revealed an antibiotic resistance. They are typically commensals of the skin and mucous membranes [16]. S. epidermidis has attracted a lot of attention in the studies since it has become one of the causative agent of nosocomial infections [17]. However, S. epidermidis classified as opportunistic because it needs a predisposed host in order to transform from

a typical resident of human skin to an infectious agent.

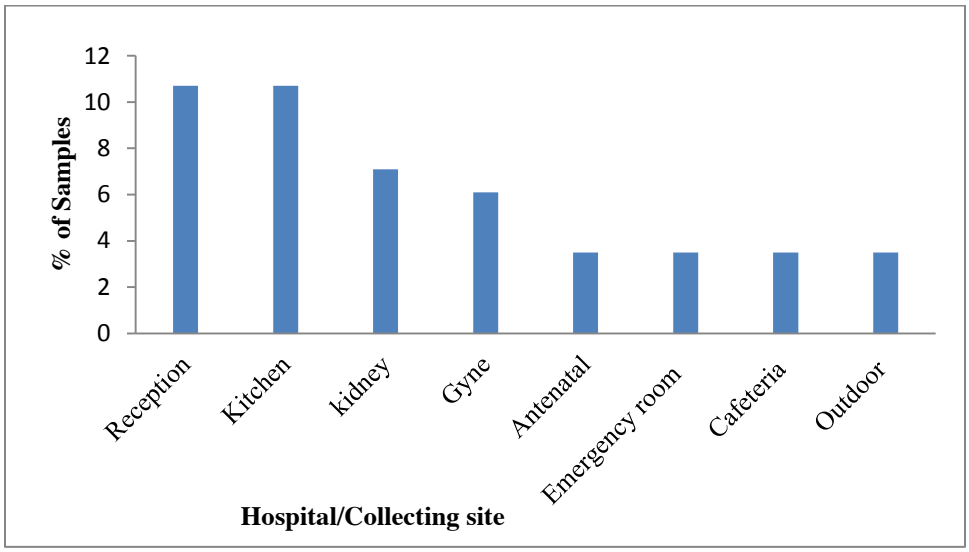

Figure 2. Percentage of bacteria from ants from hospital/collecting sites

It was observed that among nine collecting sites, the places with more frequent infestation and higher number of species of bacteria in the hospital, were the reception and kitchen, each one has 3 species (10.7%) (figure 2). Whereas the largest number of all identified bacteria, around 35.9%, was S. aureus, which was found in the antenatal.

Antibiogrm results shown in table 2 demonstrated that all of tested microorganisms were Augmentin resistant except Micrococci spp . It is very important to emphasize that the resistances found in this study include some antibiotics that are currently being used in clinical therapy in the hospitals of Libya.

The bacteria isolated form ants have a resistance profile that has been noticed [18], showing the need for conscious antibiotic use. As for the study in Bahia, Brazil, different types of ants carried different types of bacteria, ranging from gram-positive to gram-negative. Another study has demonstrated that the ant genera Pheidole, Crematogaster, Linepithema, and the species Tapinoma melanocephalum. carried both gram-positive and gram-

negative bacteria [2]. Most of the gram-positive bacteria have been sensitive to the vancomycin, cefoxitin, and oxacillin. While, most of the gram-negative bacteria have been sensitive to chloramphenicol antibiotics [2].

Table 2. Percentage of antibiotic resistance pattern of isolated bacteria
CIP=Ciprofloxacin, LEV=Levofloxacin, FOX=Cefoxitin, CN=Cephalexin, DA=Clindamycin, E=Erythromycin, AMC=Augmentin, OX=Oxacillin, ATM=Aztreonam, CAZ=Ceftazidime , TZP=Tazobactam/piperacillin , IPM=Imipenem

Gram positive bacteria									
Name of Bacteria	**Frequency**	**CIP**	**LEV**	**FOX**	**CN**	**DA**	**E**	**AMG**	**OX**
S.aureus	7	0%	0%	28%	14%	0%	43%	43%	28%
S.epidermids	6	0%	0%	50%	50%	33%	83%	100%	50%
Corynebacterium spp (diphtheroides)	10	0%	0%	0%	0%	0%	0%	10%	-
Micrococci	1	0%	0%	0%	0%	0%	0%	0%	100%
Gram negative bacteria									
Name of Bacteria	**Frequency**	**CIP**	**ATM**	**CAZ**	**TZP**	**IPM**	**CN**	**AMG**	
E.coli	1	0%	0%	0%	0%	0%	0%	100%	
Enterobacter	3	0%	0%	0%	0%	0%	0%	100%	

In present study, vancomycin as an antibiotic was not used, but we used FOX and OX, and as shown in table 2, gram-positive, S. aureus, and S. epidermidis resisted both of the antibiotics previously mentioned, especially OX (Oxacillin). Similar to the study of Ewig & Torres (2001), strains of methicillin-resistant Staphylococcus[19] were detected in this study. However, some isolated bacteria showed a multi-drug resistance (MDR) table 2.As for gram negative bacteria, a percentage of 100% resistance to Augmentin was shown and no resistance to the other antibiotics used. .

Numerous nosocomial infections are caused by gram-negative bacteria, especially in patients who are confined in hospitals for extended periods and receive intravenous drip therapy [20]. The most prevalent gram-negative bacteria in our study is Enterobacter spp., this species is highly

resistant to antibiotics which is considered a problem as Enterobacter infections can be acquired from either endogenous or exogenous sources. [21]. It can be found in the natural environment such as water, sewage, vegetables, and soil [22]. In a study established around multiple hospitals in Brazil, results revealed the presence of Enterobacter spp, and the association between Enterobacteria and ants has been observed in some Brazilian hospitals, which represents a risk to hospitalized patients [4] [9], [10].

CONCLUSION

It can be concluded that ants are may able to carry pathogenic bacteria from one place to another, with possible role of human infection. This needs to be confirmed by looking into the relationship between the bacteria carried by ants and the ones isolated from the surface in the same environment. Another relevant finding was the resistance to selected antibiotics, which increases the risk of patients, staff, and everyone in and around the hospital. So, more in depth precautionary measures need to be implemented such as washing and disinfecting hands and proper use of antibiotics.

AUTHORSHIP AND ACKNOWLEDGEMENTS

Authorship

A.K. conceived the presented idea, and worked with R.E., and N.A. to carry out the experiment, collected data, data analysis and interpretation. A.A., A,K., R,E., and N.A. contributed to sample preparation. S.G. and A.A were involved in planning and supervised the work. S.G. drafted the manuscript and revised it critically for important intellectual content; and Final approval of the version to be published. . All authors contributed to the final version of the manuscript.

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