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RESEARCH ARTICLE

PONERINE ANT (HYMENOPTERA: FORMICIDAE) AS VECTORS FOR BACTERIA IN PUBLIC HEALTH CENTRE, MADURAI, TAMIL NADU, INDIA

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ABSTRACT

Ants (Hymenoptera: Formicidae) are a highly sociable and diversified groups of insects found in tropical regions. They coexist harmoniously with humans and are well-adapted to urban environment. Several studies have found that ants can carry pathogenic microbes in their environments and cause infections / spread pathogens. The present study aimed to identify and characterize bacterial biomes presence on the body surface of the Ponerine ant, *Leptogenys chinensis*, collected from various wards at the Government Rajajii Hospital, Madurai, Tamil Nadu, India. A total of nine bacterial isolates were isolated from the external body surface of the ants, among which 55.55 % were Gram-positive bacilli (*Bacillus spp., Corynebacterium sp., and Paenibacillus spp.),* 11.11 % were Gram-positive cocci (*Staphylococcus sp.*) and 33.33 % were Gram-negative bacilli (*Pseudomonas spp.* and *Enterobacter sp.*), reportedly in hospitals around the world. The findings of the present investigation, Ponerine ants could carry Gram-positive bacilli, Gram-positive cocci and Gram-negative bacilli bacteria. This emphasizes the significance of ants as mechanical vectors for the spread of pathogens in the hospital environments.

KEYWORDS

Leptogenys chinensis; Government Rajaji Hospital; Pathogenic microorganisms; *Staphylococcus sp.*; Mechanical vectors.

1. INTRODUCTION

Ants (Hymenoptera*:* Formicidae) are social insects that are widely distributed and they are the highly diverse in tropical regions. They are coexist with humans and are easily adapted to urban environment (Beatson, 1972). According to myrmecologists, the ants serve a variety of ecological functions in ecosystems, such as nutrient cycling, soil aeration, pollination, seed dispersal, and food web maintenance. Additionally, certain species damage crops or harmful humans, which makes them highly economically significant (Della Lucia, 2003; Rust and Su, 2012; Melo et al., 2012; de Castro et al., 2015; Bitar et al., 2021).

Last four decades the ants have been serious problems in hospital environments both in respect of their prevalence and issues. Apparently, the ants enter hospital environments through food items left behind by visitors, and they establish colonies in hospitals with sterilized products **(**Bueno et al., 1999; dos Santos, 2001**)**. Ants being is a hospital environment are exposes to contaminated sites and materials, they carrying pathogenic microorganisms with them adhere to their legs and body and disseminate throughout the hospital (Alharbi et al., 2019; dos Santos et al., 2021). Additionally, the scientists found evidence of multidrug-resistant bacteria strains in ants from different hospitals around the world, which could lead to serious public health problems in hospitals in the future (Peçanha, 2000; dos Santos et al., 2021; Rawat et al., 2023).

The first reports of ants causing infections in hospitals in Europe and Brazil raised interest in this subject (de Castro et al., 2015). There has been evidence that human pathogenic microorganisms can be transmitted by ants in hospitals in several countries, including United Kingdom, Chile, Germany, Trinidad, Spain, Japan, Colombia, Korea, United States, Philippines, Iran, Nigeria, Mauriti, Kuwait and so on (Edward and Backer, 1981; Ipinza-Regla et al., 1981; Eicheler, 1990; Chadee and Maitre, 1990; Espalder and Espejo, 2002; Yomoda et al., 2003; Olaya-Masmela et al., 2005; Kim et al., 2005; Nelder et al., 2006; Bandoy and Tiu, 2017; Shahi et al., 2017; Ogba et al., 2017; Simothy et al., 2018; Alharbi et al., 2019). Based on a literature review survey, no study has yet been conducted on ants as disease vectors in India.

It has been reported that a variety of pathogens have been isolated from ants species including *Monomorium pharaonis* [e.g., *Salmonella* sp., *Pseudomonas æruginosa*, *Staphylococcus* spp., *Streptococcus* sp., *Clostridium* sp., etc.], *Tapinoma melanocephalum* [e.g., *Staphylococcus* sp., *Enterococcus* sp., *Sphingomonas paucimobilis* etc.], *Camponotus vittatus* [e.g., *Staphylococcus* sp. -], *Wasmannia auropunctata* [e.g., *Pseudomonas aeruginosa, Staphylococcus* sp., *Streptococcus* sp. etc. -], *Linepithema humile* [e.g., *Escherichia coli, Streptococcus* sp. etc. -], *Solenopsis saevissima* [e.g., *Corynebacterium* sp., *Enterococcus* sp., *Neisseria* sp., *Pseudomonas luteola* etc. -, *Paratrechina longicornis* [e.g., *Acinetobacter haemolyticus* -] etc in hospital environments (Beatson, 1972; Lise et al., 2006; do Nascimento et al., 2020; Lise et al., 2006; dos Santos et al., 2009; Rodovalho et al., 2007; dos Santos et al., 2009; dos Santos et al., 2009; Lise et al., 2006; Lise et al., 2006). The association between ants and bacteria has raised concerns about the potential for ants to act as disease vectors (de Castro et al., 2015; Rajagopal et al., 2019).

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In this perspective, the objective of this study was to determine the bacterial biomes of the exterior body parts of Ponerine ants (*Leptogenys chinensis*), which were collected from the Government Rajaji Hospital in Madurai, Tamil Nadu. This ant is fairly common in the Government Rajaji hospital environment. To our knowledge, this is the first such study performed in a public health centre in the state of Tamil Nadu, India.

2. Materials and Methods

2.1 Study site

The study was conducted at the Government Rajaji Hospital (GRH), Madurai, is one of the most renowned Public Health Centre in the area situated at Goripalayam (9.9281° N, 78.1295° E), Madurai, Tamil Nadu. The GRH is one of the oldest hospitals in India, established in 1842 on a total of 12 acres of land. It is easily accessible through various modes of transport. Providing the highest standard of care for both, minor and major health issues, hospitals create a safe space for patients by offering end-to-end clinical, surgical, and diagnostic services. The ant specimen was collected from various wards of GRH *viz*., reception area, medical wards, and paediatric clinic.

2.2 Collection and identification of ants

Ant specimens were collected in different wards of GRH and were placed in a separate vials containing 70% alcohol and transported to the Entomology Laboratory for ant identification. In addition, the ant's species identification was performed based on the identification keys proposed by (Bolton, 1975; 1995). The collected ant's specimens were kept in the Thiagarajar College Zoological Museum for the benefit of the students' future research. A total of 30 Ponerine ants (*Leptogenys chiensis*) were obtained from in different wards (10 ants in each ward) for microbiological analysis.

2.3 Isolation and characterization of bacteria

The ants were randomly picked from containers with the help forceps and placed into sterile dilution test tube containing peptone water and added ten milliliters of sterile normal saline (0.9%) to test tubes, were thoroughly shaken for 2 min to isolate microorganisms from the external surface. Serially diluted (10^{-1} to 10^{-7}) 0.1 ml aliquots were then separately inoculated onto nutrient agar plates and incubated overnight at 37 °C. Further, isolated colonies were taken from the culture plate and it was streaked on a fresh plate containing nutrient agar and the pure culture was obtained.

Bacterial colonies were initially identified by morphological appearance, microscopic examination using staining techniques, and identified further by biochemical tests *viz*., gram staining, motility test, indole test, methyl red test, vogesproskauer test (acetoin production), citrate utilization test, hydrogen sulfide (H_2S) production test, triple sugar iron test, catalase test, oxidase test, starch hydrolyses test, protease production test, urease test and carbohydrate fermentation test (glucose, maltose and sucrose). The methodology used in the microbiological analyses followed the standards of the microbiology laboratory manual described (Cappuccino and Sherman, 1999).

3. Results

The ants collected from GRH, Madurai were identified as *Leptogenys chinensis* based on the following combination of characters: (i) Petiolar node moderately to weakly elongate in lateral view, less than 1.2 times as long as high (ii) Clypeus truncated at apex, and (iii) Anterior clypeal margin distinctly laterally sinusoid; propodeal declivity transversely striate. Nine bacterial isolates were isolated from the external body surface of *Leptogenys chinensis* using nutrient agar medium and are provided with the sample codes H1 to H9. The isolate H1 is brown, rod shape and motile; the H2, H5, H6, H8 and H9 isolates were white milky texture, rod shape and motile, H3 is orange, *cocci*, non-motile, H4 is yellow, rod shape and non-motile and H7 isolate is yellow, rod shape, non- motile bacteria. Gram stain determination showed that six isolates had gram positive bacteria (H1, H3, H5, H6, H8 and H9) while H2, H4, H7 isolates was gram negative. Among the isolated bacteria, 55.55 % were Gram-positive *bacilli*; of these 11.11 % were Gram-positive *cocci* and about 33.33 % were Gram-negative *bacilli* (Table 1; Figure 1).

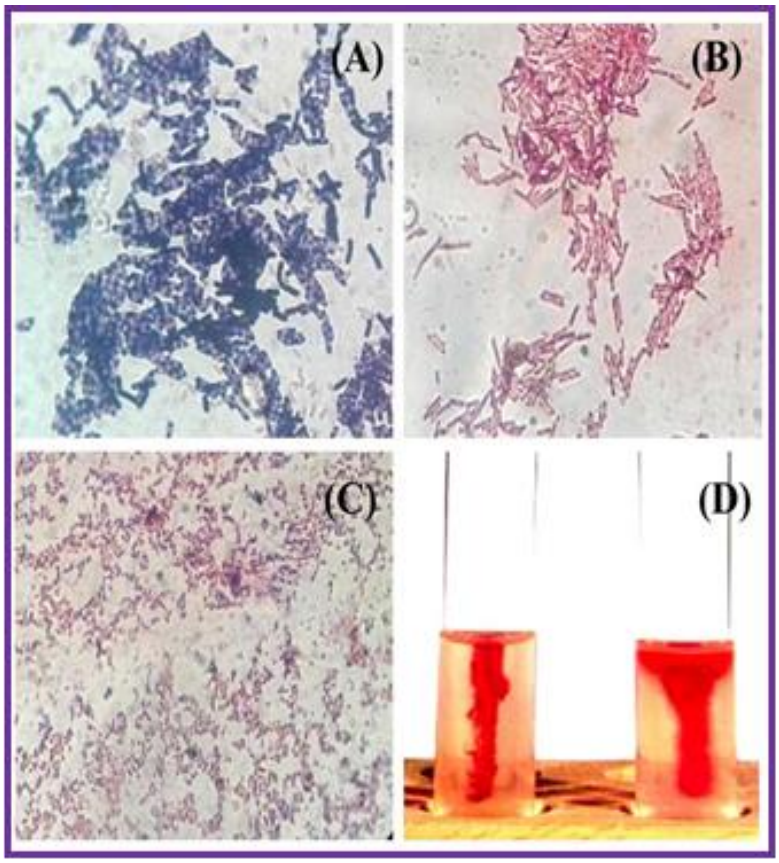

Figure 1: Morphological analysis of bacterial isolates: (A) Gram positive rod, (B) Gram positive cocci, (C) Gram negative rod and (D) Motility test.

Based on morphological appearance and biochemical characterization, the isolates H1 and H9 were determined to belong to the bacterial genus *Bacillus* and have been classified as *Bacillus* sp1 and *Bacillus* sp2 respectively. The contents of isolate H2 as *Pseudomonas putida*, H3 as *Staphylococcus* sp., H4 as *Enterobacter cloacae*, H5 as *Corynebacterium* sp., H6 as *Bacillus licheniformis*, H7 as *Pseudomonas* sp1 and H8 was *Paenibacillus pectinilyticus*. A total of nine bacterial strains were isolated, of which 33.33% were *Bacillus spp*., 22.22 % *Pseudomonas spp.*, 11.11 % each of *Staphylococcus* sp., *Enterobacter* sp., *Corynebacterium* sp., and *Paenibacillus* (Table 1; Figure 2).

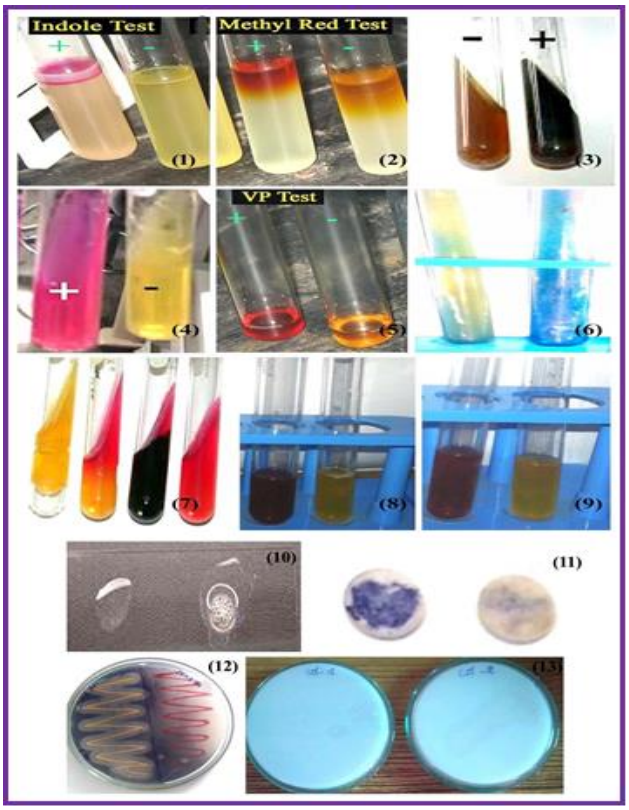

Figure 2: Biochemical analysis of bacterial isolates: (1) Indole test, (2) Methyl red test, (3) H2S production test, (4) Urease test, (5) Voges - Proskauer test, (6) Citrate utilization test, (7) TSI test, (8) Fermentation test - gas production and (9) Fermentation test – acid production, (10) Catalase test, (11) Oxidase test, (12) Starch hydrolysis test and (13) Protease test.

Table 1: Morphological and Biochemical analysis of bacterial isolates.

S.No.	Test			Bacterial Isolates								
				H1	H2	H3	H4	H5	H6	H7	H8	H9
Morphological Analysis												
1	Gram staining			+	-	+	-	+	+	-	+	+
2	Shape			Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Rod	Rod
3	Motility test			+	+	-	+	+	+	-	+	+
Biochemical Analysis												
4	Indole test			-	-	-	-	-	-	-	-	-
5	Methyl red test			+	+	+	+	+	+	+	+	+
6	Voges-proskauer test			+	+	+	+	+	+	+	+	+
7	Hydrogen production test			+	+	+	-	+	-	-	-	-
8	TSI Test (H_2S)	Acid/Acid reaction		+	+	+	-	+	-	-	-	-
		Alkaline/Acid reaction		+	+	+	+	+	+	-	+	-
9	Catalase test			+	+	+	+	-	+	-	+	-
10	Citrate utilization test			+	+	+	+	+	+	+	+	+
11	Oxidase disc test			+	+	-	+	+	-	+	+	+
12	Starch hydrolysis test			+	-	+	+	+	+	+	-	-
13	Protease test			-	-	+	-	+	+	+	-	+
14	Urease test			+	+	+	+	-	-	-	+	+
15	Carbohydrate Fermentation Test	Glucose	Acid	+	+	+	+	+	+	+	+	+
			Gas	-	+	+	+	+	-	+	-	-
		Maltose	Acid	+	-	+	+	+	+	+	-	+
			Gas	-	-	-	-	+	-	+	-	-
		Sucrose	Acid	+	-	+	+	+	+	+	+	+
			Gas	-	-	-	-	+	+	+	+	+

Organism: H1 – *Bacillus* sp1.; **H2** - *Pseudomonas putida*; **H3** - *Staphylococcus* sp.; **H4** - *Enterobacter cloacae*; **H5** - *Corynebacterium* sp.; **H6** - *Bacillus licheniformis*; **H7** - *Pseudomonas* sp1.; **H8** - *Paenibacillus pectinilyticus*; **H9** - *Bacillus* sp2.

4. Discussion

Ants are mechanical carriers of pathogenic microorganisms, they can spread microbes from one area of the hospital environment to another (Bueno, 1995; Lise et al., 2006). According to the ants act as vectors of bacteria such as *Enterococcus spp*., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter spp*., *E. coli*, *Klebsiella spp*., and *Enterobacter spp*., etc., in hospital environments and cause infections where they are found (Oliveira et al., 2017; do Nascimento et al., 2020). In the present study, nine bacterial strains were found to be isolated in Ponerine ant, of them, Gram-positive *bacilli* were the most abundant species followed by Gram-negative *bacilli* and Gram-positive *cocci*. Similar findings have been found on *Paratrechina* and *Monomorium* ant's species, they had a higher capacity for carrying Gram-positive, spore- producing bacilli with 68.80% than Gram-negative bacilli with 14.70% and Gram-positive *cocci* with 6.40% in the hospital environments (Maximo et al., 2014). According to previous research, the Gram-positive bacilli species' remarkable ability to form endospores allows them to flourish in areas with extreme environmental instability (Chadee and Maitre, 1990; Boursaux-Eude and Gross, 2000).

Based on microbiological investigation, the isolates H1, H6 and H9 were characterized as *Bacillus* spp., these bacteria has been identified in a variety of invertebrates' species and form a significant portion of the intestinal microbial community (Kuhnigk and Konig, 1997; Drake et al., 2005). There is a report that *Bacillus* species commonly found in some ant species like *Tapinoma melanocephalum*, *Monomorium Pharaonis*, *Tapinoma melanocephalum*, *Solenopsis saevissima* etc., were collected in hospital environments around the world (Bandoy and Tiu, 2017; Moreira et al., 2005; Bandoy and Tiu, 2017; do Nascimento et al., 2020; Moreira et al., 2005). The present findings suggest that the *Bacillus* spp. which may be able to contribute to the degradation of biological materials such as chitin, cellulose, polysaccharides, aromatic compounds etc in their habitats.

Pseudomonas species were found in 22.22% of the Government Rajaji hospital environment in Madurai. *Pseudomonas* spp. are commonly found in soil, water and insect. They are metabolically versatile and may adapt to a variety of habitats and nutritional conditions (Fernandez et al., 2009; Dogan et al., 2011). Infections by *Pseudomonas species* have been reported to be linked to insertion of catheters or drainage tubes etc (Yoshino et al., 2011). A strain of *Staphylococcus* sp. was isolated in Government Rajaji hospital environment. The *Staphylococcus species* can lead to high levels of contamination and cause diseases related to some domestic animals that harbor this bacterium in their fur, in addition to contaminating food scraps and causing several food-borne infections (Hirsh, 2003; Szweda et al., 2012). Trabulsi and Toledo reported that *Staphylococcus spp*., can cause infections including pharyngitis, glomerulonephritis, rheumatic fever, septicemia etc (Trabulsi and Toledo, 1991). Additionally, *Staphylococcus bovis* is typically found in the human intestine and which causes urinary infections and endocarditis (Trabulsi and Toledo, 1991; Rodovalho et al., 2003).

Enterobacter species are motile aerobic gram negative bacilli belonging to the family *Enterobacteriaceae*. Fridkin reported *Enterobacter* as the fifth leading cause of ICU infections in the United States and the third most common cause of nosocomial pneumonia (Fridkin, 2001). Several outbreaks have been documented in *Enterobacter*, including contaminated enteral feeding area, humidifiers and respiratory therapy equipment, hydrotherapy water in a burn unit etc (Simmons et al., 1989; Wang et al., 1991; Mayhall et al., 1979). Additionally, this bacterium is well adapted to different environmental conditions and can survive on skin and dry surfaces as well as replicate in contaminated fluids (Fridkin, 2001). In the present study, the Ponerine ants were found to carry *Enterobacter*, which can lead to nosocomal infection at Government Rajaji hospital in Madurai, Tamil Nadu.

In the present investigation, bacteria *Corynebacterium* sp. and *Paenibacillus* sp. were isolated from the body surfaces of Ponerine ants. The genus *Corynebacterium* is a member of the *Actinobacteria,* which comprises a collection of morphologically irregular or club-shaped non-sporulating aerobic microorganisms. To date, 90 *Corynebacterium* species have been classified by microbiologist, of whom 52 species are sporadic or rare sources of infections, while only a few cause severe infections (Sangal et al., 2014). There have been reports that several species *Paenibacillus* bacteria can cause bacteremic infections in humans. For example, *Paenibacillus thiaminolyticus* is a cause of bacteremia in hemodialysis patients, *Paenibacillus konsidensis* is a cause of bacteremia in febrile patients with hematemesis, *Paenibacillus alvei* is a cause of bacteremia in prosthetic joint infection etc (Ouyang et al., 2008; Ko et al., 2008; Reboli et al., 1989). Roux and Raoult isolated three novel *Paenibacillus* bacterial species (*i.e.*, *Paenibacillus massiliensis*, *Paenibacillus sanguinis* and *Paenibacillus timonensis*) from blood cultures of patients with carcinoma, interstitial nephropathy, and leukemia, respectively (Roux and Raoult, 2004). The present investigation demonstrated that the Ponerine ants can carry the pathogenic bacteria like *Paenibacillus* sp. and *Corynebacterium sp.*, which could spread infection to patients in hospital environments.

5. Conclusion

This is the first report as far as isolation of bacterial biomes on the surface of ant bodies obtained from public health centre. The nine bacterial strains were isolated from the ponerine ants in the government Rajaji hospital environments showed that ants are potential carriers of pathogenic and opportunistic bacteria, which represent risk factors for infections in hospital environments. Additionally, this finding emphasizes the necessity of increased awareness in public health centre regarding strict preventative measures, notably managing ants population in healthcare centre. Future research is required to investigate the genome sequencing in the isolated bacteria in order to confirm the species level.

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