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Habitat influences on diversity of bacteria found on German cockroach in Beijing

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Abstract

Cockroaches are worldwide indoor pests carrying microorganisms of medical importance. German cockroaches (*Blattella germanica*) were sampled in five habitats (hospital, restaurant, office home, and market) in Beijing, and the bacteria were isolated from their external surface and alimentary tract and identified using a Biolog identification system. Cockroach densities significantly differed among habitats (market > home > office > restaurant > hospital). However, no significant differences in bacterial abundance carried by individual German cockroaches (of either sex) were found among habitats. The bacterial abundance in the gut was significantly higher than that on the surface. There were no significant differences in bacterial species richness observed among habitats, sex, carrying position or their interaction. Cluster analysis showed that cockroach densities and bacterial abundance found in the market differed significantly from the other four habitats. The bacterial diversity was not significantly reduced in sensitive facilities such as hospital and restaurant, even though pesticide and bactericide were more frequently applied there. The implications of these findings were discussed in this article.

Key words: German cockroaches; *Blattella germanica*; diversity; bacteria
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Introduction

Cockroaches are omnivorous (Bennett, 1993) and vectors of bacteria, fungi, viruses, and parasites (Bracke *et al*., 1979; Fotedar *et al*., 1989; Graczyk *et al*., 2005; Lemos *et al*., 2006; Salehzadeh *et al*., 2007). Some bacterial species carried by this urban indoor pest are medically important (Cloarec *et al*., 1992; Pai *et al*., 2004). Thus, cockroaches are a major target pest of urban pest management. Many methods, including insecticide applications, are used to manage cockroach infestations. However, management is difficult because cockroaches are notoriously resilient and readily infest diverse habitats (Service, 1996). All habitats in the urban ecosystem should be considered together to manage cockroaches using integrated pest management (IPM) (Schal and Hamilton, 1990) because piecemeal solutions often do not work (Ebesu, 2003). Understanding the influence of habitats on species diversity of bacteria carried by cockroaches is an important step toward develop an effective cockroach integrated pest management (IPM) strategy.

The species and population abundance of bacteria carried by the German cockroach vary in different habitats. Burgess (1984) found that cockroaches in hospitals, restaurants, hotels and private homes could harbor pathogenic organisms. Chaichanawongsaroj *et al*. (2004) surveyed the pathogenic Gram-negative bacteria carried on the cuticles of cockroaches in hospitals, food-handling establishments, and human dwellings in Thailand. However, information on species and strains of pathogenic bacteria carried by cockroaches in various habitats is limited (He *et al*., 1992; Guo *et al*., 1999).

German cockroach (*Blattella germanica*), is one of the most important pests in Beijing and accounts for 96.9% of all cockroach species in the city (Yan *et al*., 2005). Cockroaches occur in different habitats, such as hospital, restaurant, office, home, and market, in the urban community together with the bacteria they harbor. However, the species and abundance of bacteria carried by them in different habitats in Beijing are not well known.

In this article, the study on the diversity of bacteria carried by German cockroach in an urban community and the relationships among the bacterial flora carried by cockroaches in different habitats was conducted to address three questions: (1) What bacterial species are carried by German cockroaches in Beijing? (2) What are the similarities and differences in abundance and diversity of bacteria carried by German cockroaches in different habitats? (3) How does one develop efficient urban IPM strategies for

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the cockroaches and pathogens if the bacterial species, abundance and distribution are known?

1 Materials and methods

1.1 Cockroach collection

The investigation was carried out in Chaoyang District, Beijing, in different habitats located in one community. We selected five different representative habitats including hospital, restaurant, office, dwelling house, and market.

German cockroaches were sampled continuously from five habitats from 20 June to 20 July, 2006. Three traps were placed in each habitat (Jeffery *et al*., 1984). Adult German cockroaches were collected daily from each trap. Cockroach sex was determined by presence/absence of male styli on the ninth abdominal sternum. Roaches were then placed separately in sterile tubes for later research. Altogether 55 roaches were randomly selected for later research from all the five habitats.

1.2 Microorganism isolation and identification

Each tube containing a cockroach was frozen for 5 min, then 2 mL of sterile normal saline was added, followed by vigorous shaking for 2 min, the bacterial suspension formed from the external surface (ES). The roach was dipped in 70% ethanol for 5 min and washed with sterile saline for 3 min, followed by drying at room temperature under sterile conditions. Gut dissection was conducted with sterile appliances and the gut was triturated with a mortar and pestle after 2 mL of sterile normal saline was added to the mortar which was the suspension from the alimentary tract (AT).

The bacterial suspension (0.2 mL) from the ES and AT was inoculated onto the plate of Biolog Universal Growth Agar (Biolog Inc., Hayward, USA) and incubated at 35°C for 24 h.

Colonies on each plate were counted and representative ones were chosen for isolation and purification. Each isolated pure strain was identified by Gram-staining and Biolog identification system (Biolog Inc., USA).

1.3 Data analysis

Calculations using Shannon-Wiener's diversity index (H′) (Shannon and Wiener, 1949) were conducted to measure the bacterial community diversity (all species of bacteria) in each habitat:

$$H' = -\sum P_i \ln P_i \quad (1)$$

$$P_i = n_i/N \quad (2)$$

where, P_i is the proportion of the ith bacterial species in the total sample, N is the number of species richness.

Jaccard's index of similarity was used to analyze the similarity of the bacterial community among habitats (Jaccard, 1912).

$$p = c/(a + b - c) \quad (3)$$

where, p is Jaccard's index of similarity, c is the number of the shared bacterial species, a is the number of species in one habitat, b is the number of species in another habitat.

The number of colony forming units found from the cuticle and the gut of each cockroach was compared using multiple ANOVA (MANOVA). Rank correlation analysis was used to determine whether the abundance of bacteria on the external surface correlated to that in the alimentary tract. The frequencies of each kind of bacteria isolated from the ES and AT sites were compared using x^2 and Fisher's exact test, as appropriate.

Spearmans test of rank correlation was used to compare the ES and AT bacterial species in each of the five habitats. The bacterial species isolated from the gut, surface, and total number in different habitats were compared using separate Chi-square tests. Using Jaccard's index of similarity and the unweighted arithmetic average sorting strategy, cluster analysis was applied to classify habitats (Yuan and Liu, 2000). The data were standard transformed when necessary. Quantitative data analysis was performed with SPSS 13.0.1 for PC (SPSS Inc., IL, USA).

Male and female cockroaches were not significantly different in bacterial abundance or species represented, and these data were pooled for further analysis.

2 Results

2.1 Infestation of cockroaches and microbes

Altogether 72, 114, 266, 354, 510 of cockroaches were captured from hospital, restaurant, office, dwelling house, and market, respectively. The cockroaches captured per trap from hospital and restaurant were significantly less than those from the dwelling and office. The highest density of cockroaches occurred in the market (Fig. 1).

Bacterial density per cockroach did not differ by sex or among habitats, or their interaction (Table 1). Roaches did have significantly more abundant bacteria internally than externally ($df = 1$, $F = 96.338$, $P = 0.000$). The bacterial abundance detected on the pest surface varied from 0 to 1.2×10^6 per cockroach and the number from the gut ranged from 8.9×10^3 to 1.4×10^7 per cockroach. Correlation analysis showed that relative surface and gut bacterial abundance were consistent for each cockroach, i.e., a roach with high density on the surface would also have high density in the gut (Spearman correlation coefficient = 0.531, $P = 0.000$).

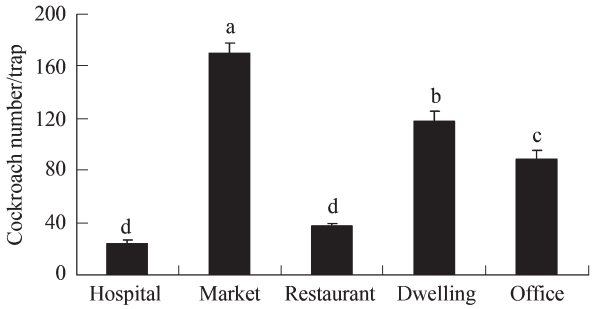

Fig. 1 The number of cockroaches captured per trap during 20 June to 20 July 2006 in different habitats in Beijing, China.

Table 1 Bacterial abundance and species richness per cockroach using MANOVA (n = 51)

Source	Bacteria			Abundance		
	df	F	P	df	F	P
Habitat	4	1.840	0.128	4	0.491	0.743
Sex	1	0.155	0.695	1	2.661	0.107
Carrying position	1	99.734	0.000	1	0.426	0.516
Habitat vs. sex	4	1.553	0.194	4	0.610	0.657
Habitat vs. carrying position	4	0.587	0.673	4	0.978	0.424
Carrying position vs. sex	1	0.007	0.933	1	0.532	0.468
Habitat vs. carrying position vs. sex	4	1.925	0.113	4	1.028	0.398

2.2 Isolation of microorganisms

In this study, 51 cockroaches out of the 55 from traps were observed for the presence of bacterial flora and 387 strains of bacteria were obtained. Total 28 genera and 55 species, including 32 species of Gram-positive bacteria and 23 species of Gram-negative bacteria, were isolated from the cuticles and the guts of the German cockroaches in five habitats (Tables 2 and 3). No significant difference of the positive rate was found between the external surface and the alimentary tract for the Gram-positive species richness (χ^2 = 0.00, df = 1, P > 0.05) (Table 2). However, there was significantly lower Gram-negative species richness externally than internally (χ^2 = 13.95, df = 1, P < 0.001) (Table 3).

Table 2 Gram-positive bacterial species and frequency of isolation from *B. germanica* collected from five habitats in Beijing (n = 51)

Bacterial species	Number from ES (%)	Number from AT (%)	P
A. bernardiae	1 (1.96)	0	NS
A. cumminsii	12 (23.53)	6 (11.76)	NS
B. amyloliquefaciens	0	1 (1.96)	NS
B. badius	4 (7.84)	1 (1.96)	NS
B. cereus	7 (13.73)	3 (5.88)	NS
B. licheniformis	1 (1.96)	2 (3.92)	NS
B. maroccanus	0	2 (3.92)	NS
B. megaterium	2 (3.92)	0	NS
B. myoides	2 (3.92)	1 (1.96)	NS
B. pumilus	15 (29.41)	9 (17.65)	NS
B. subtilis	7 (13.73)	10 (19.61)	NS
C. cellulans	1 (1.96)	1 (1.96)	NS
C. agropyri	5 (9.80)	4 (7.84)	NS
E. avium	1 (1.96)	3 (5.88)	NS
E. durans	1 (1.96)	1 (1.96)	NS
E. faecalis	12 (23.53)	13 (25.49)	NS
E. raffinosus	3 (5.88)	7 (13.73)	NS
G. thermoglucosidasius	3 (5.88)	0	NS
K. sibirica	4 (7.84)	0	NS
L. grayi	2 (3.92)	2 (3.92)	NS
L. monocytogenes	1 (1.96)	0	NS
M. bovicus	1 (1.96)	1 (1.96)	NS
M. caseolyticus	3 (5.88)	0	NS
M. equipercicus	1 (1.96)	1 (1.96)	NS
M. luteus	25 (49.02)	19 (37.25)	NS
M. lylae	3 (5.88)	3 (5.88)	NS
R. rhodnii	0	3 (5.88)	NS
S. lentus	5 (9.80)	1 (1.96)	NS
S. pasteuri	7 (13.73)	3 (5.88)	NS
S. saprophyticus	6 (11.76)	2 (3.92)	NS
S. sciuri	2 (3.92)	2 (3.92)	NS
S. gordonii	1 (1.96)	1 (1.96)	NS
Overall[a]	47(92.16)	47(92.16)	NS

NS: not significant (P > 0.05).
[a] One cockroach may harbor more than one species of bacteria.

Table 3 Gram-negative bacteria species and frequency of isolation from *B. germanica* collected from five habitats in Beijing (n = 51)

Bacterial species	Number from ES (%)	Number from AT (%)	P
A. haemolyticus/ *genospecies* 4*	1 (1.96)	1 (1.96)	NS
A. genospecies 15*	1 (1.96)	1 (1.96)	NS
A. radioresistens/ *genospecies* 12*	4 (7.84)	2 (3.92)	NS
B. caryophylli	2 (3.92)	4 (7.84)	NS
C. freundii	11 (21.57)	13 (25.49)	NS
C. koseri	5 (9.80)	9 (17.65)	NS
E. aerogenes	0	3 (5.88)	NS
E. asburiae	1 (1.96)	1 (1.96)	NS
E. cancerogenus	0	1 (1.96)	NS
E. cloacae	0	3 (5.88)	NS
F. mizutaii-like	7 (13.73)	6 (11.76)	NS
K. pneumoniae	0	1 (1.96)	NS
M. canis	1 (1.96)	2 (3.92)	NS
O. proteus	1 (1.96)	5 (9.80)	NS
P. dispersa	1 (1.96)	3 (5.88)	NS
P. stewartii	9 (17.65)[a]	22 (43.14)	0.000
P. lundensis	0	1 (1.96)	NS
P. tolaasii	0	2 (3.92)	NS
R. terrigena	2 (3.92)	3 (5.88)	NS
S. gp1. ST gallinarum*	0	2 (3.92)	NS
*S. gp6**	0	1 (1.96)	NS
S. marcescens	4 (7.84)	7 (13.73)	NS
S. sanguinis	0	2 (3.92)	NS
Overall[b]	31 (60.78)[a]	47 (92.16)	0.000

* The numbers indicate the type of the bacterium.
[a] External surface vs. alimentary tract: P < 0.05; [b] one cockroach may harbor more than one kind of bacteria.
NS: not significant (P > 0.05).

The dominant species in the five habitats were *Micrococcus luteus*, *Pantoea stewartii*, *Enterococcus faecalis*, and *Bacillus pumilus*. *Salmonellae* and *Listeria monocytogenes* were found in our studies. Unexpectedly, *Escherichia coli*, as the most common potentially-pathogenic bacteria, was not detected in our investigation.

Altogether, 28, 29, 28, 29, and 37 species of bacteria were detected on cockroaches collected from hospital, restaurant, office, home, and market, respectively. No significant differences in bacterial species richness on individual cockroaches were observed among habitat, sex, or carrying position (Table 1). The highest Shannon-Wiener's diversity index value was found in bacteria on cockroaches from the market (Table 4). Jaccard's index of similarity of bacteria species in both carrying positions was the smallest for cockroaches collected from the market (Table 4).

A significant (P < 0.01) correlation in bacterial species found on the external surface and in the gut of cockroaches occurred in four habitats, but not in the market (P >

0.05). No significant difference was found among the total bacterial species isolated from the cockroach gut in five habitats (χ^2 = 0.220, df = 1, P > 0.05), while there were more species isolated externally from the cockroach in the market than in the dwelling house (χ^2 = 6.565, df = 1, P < 0.05) and the office (χ^2 = 4.591, df = 1, P < 0.05). The cockroach bacterial species richness in the hospital and the restaurant was not significantly different from that of the dwelling house and the office (Table 5). Taking bacterial species and similarity index as markers, after standardization, the five habitats were grouped into two parts with the market treated separately (Fig. 2).

Table 4 Community diversity indices for bacterial species in different habitats

Habitat	Species	Jaccard's index of similarity		Shannon-Wiener's diversity index
		Among habitats	Between ES and AT	
Hospital	28	0.418	0.536	3.01092
Restaurant	29	0.491	0.414	3.18129
Office	28	0.509	0.429	3.14218
Dwelling house	29	0.491	0.345	3.08614
Market	37	0.582	0.324	3.40262

Table 5 P value in χ^2 test of the bacterial species between two habitats per cockroach

	Hospital	Restaurant	Office	Dwelling house	Market
Hospital	/	0.805	1.000	0.820	0.044*
Restaurant	/	/	0.805	0.191	0.078
Office	/	/	/	0.820	0.034*
Dwelling house	/	/	/	/	0.057
Market	/	/	/	/	/

* P < 0.05.

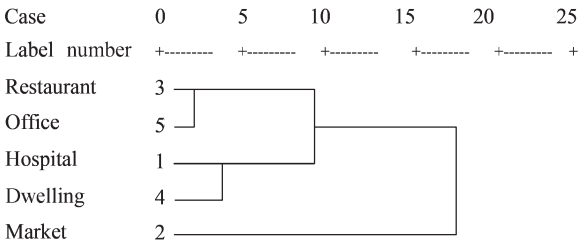

Fig. 2 Cluster analysis of habitats.

3 Discussion

The five habitats in this study represent typical urban sites where human-roach contact may occur, and collectively represent an environment where an IPM strategy could be developed. Considering them as a whole unit, instead of studying them independently, allows one to consider if and how cockroach infestation and invasion might play an important role in the kind and incidence of bacteria carried by the cockroaches.

Bacteria (55 species) were isolated from the German cockroaches collected from the five habitats studied. These cockroaches harbored 18 bacterial species known to be pathogenic or potentially-pathogenic. An investigation in Libya reported 27 species of potentially pathogenic bacteria (Elgderi *et al*., 2006). Chaichanawongsaroj *et al*. (2004) observed that the Gram-negative dominant species were *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. in hospitals, and *E. coli*, *Klebsiella* spp. and *Citrobacter* spp. in the dwellings and food-handing establishments. However, our research showed that the dominant species were *M. luteus*, *E. faecalis*, and *B. pumilus*, which should belong to the dominant airborne bacterial genus (Fang, 2006). Although *E. coli* exists widely in intestines of man and other homeotherms, few was found in the cockroach in works by Pai *et al*. (2005) and Elgderi *et al*. (2006). In this study, *Salmonellae* spp. and *L. monocytogenes*, which are important food borne pathogenic bacteria, were detected several times, while *E. coli* was not found.

Our results showed that there was no significant difference in the positive rate between the external surface and the alimentary tract for the Gram-positive bacterial species. However, for the Gram-negative species, the positive rate on the surface was significantly lower than that in the tract. These results were consistent with Pai's study (Pai *et al*., 2005). This could be explained by the enteric Gram-negative bacteria being better adapted to infest in the gut, where the stable and nutritious conditions are superior to those on the exterior.

Shannon-Wiener's diversity index was the highest for the market habitat. Jaccard's index of similarity for bacterial species found externally and internally was the smallest in the market habitat, showing that the bacterial species in the gut differed from those on the surface. The lack of correlation of the bacterial species between the external surface and internal tract of cockroaches in the market habitat also suggests that the bacterial species in the gut exist somewhat independently relative to those on the surface. In the cluster analysis, the market was clustered into a specific group, which meant a different character of bacterial flora. In this community market, all commodities come daily from a suburban wholesale product market by van, which provides a conduit for pests to initially enter this community. Although periodic pest-killing activity is carried out, the invaders colonize and infest in the market where food and shelter are always abundant. With lower selection pressure comparatively, or with less disturbance, bacterial community in the pest alimentary tract could infest stably.

In restaurant and hospital, pesticide is always placed everywhere, and bactericide is sprayed frequently. After a long-term bactericide selection, species of common pathogenic bacteria isolated from cockroaches showed resistance to many antibiotics (Pai *et al*., 2004; Mpuchane *et al*., 2006). Antibiotic disturbance could influence the bacteria community structure not only on the external

surface but also in the alimentary tract from time to time, which would lead to the similar development tendency of bacterial community structure. The total numbers of bacterial species in the hospital and restaurant had no significant difference from those of the dwelling house and office, which indicates that higher selection pressure from the insecticide and bactericide in more sensitive areas could not inhibit bacterial species on cockroach successfully in the end, even though the cockroaches were killed a lot temporarily there. In other words, the quantity of the bacteria carried by the cockroach population was inhibited because of the decline of cockroach population, which meant that the measures had some effect on the bacteria amount instead of restraining the bacteria species. Habitats such as medical facilities or food preparation areas are related closely to the outdoor circumstances such as markets which supply the continuous assistance of cockroaches and potentially harbors and disperse pests. Cockroach density indoors can resurge after management due to immigration and dissipation of residues. Dispersal of this pest in the community may occur via many media, i.e., water or steam pipes, which might serve as migration routes from room to room (Graczyk *et al*., 2005). The relative abundance of bacteria on resident cockroaches in these habitats might be a transient reservoir that could lead to a rapid recovery of bacteria flora with the pest population infestation in the near future (Hossein *et al*., 2003). Therefore, preventing cockroaches from entrying into a given facility (for example, roaches can be carried into the home in grocery bags, in cardboard boxes, and even in food containers) is an important step in controlling the pest (Baumholtz *et al*., 1997).

Our results suggest that sensitive areas such as medical institutes should follow the principle of IPM, which advocates managing the bacteria and their pest vectors, hospitals and their surrounding habitats as a whole. Sanitation with physical barriers and careful inspection of materials being transported into sensitive areas for roaches are key elements to reduce immigration. Then, bactericide and insecticide should be used simultaneously and consistently to reduce roach and bacterial densities in sensitive facilities; on the other side, hygiene department of a city should enhance the management in the circulating sites such as markets and stations that serve as an external source of bacteria and pests for a whole town through daily importing of materials from these circulating sites to those sensitive areas.

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