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Cockroach Microbiome Disrupts Indoor Environmental Microbial Ecology with Potential Public Health Implications

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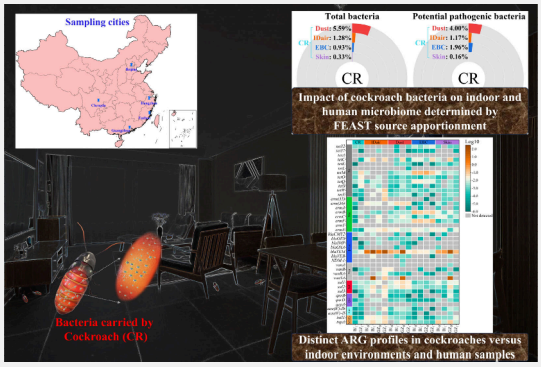
ABSTRACT: Cockroaches pose a significant global public health concern. However, besides the well-recognized cockroach-induced allergy, the potential impact of the cockroach microbiome on human health through various means is not yet fully elucidated. This study aimed to clarify the health impacts of cockroaches by investigating the microbial interactions among cockroaches, the indoor environment, and humans. We simultaneously collected cockroach, indoor environment (indoor air and floor dust), and human (exhaled breath condensate and skin) samples from residential areas in five cities representing distinct climate zones in China. The 16S rDNA sequencing results revealed that cockroaches harbor diverse bacterial populations that vary across different cities. The prevalence of potential pathogenic bacteria (PPB) in cockroaches ranged from 1.1% to 58.9%, with dominant resistance genes conferring resistance to tetracycline, macrolide, and beta-lactam. The relationships between the cockroach microbiome and the associated environmental and human microbiomes were explored by using fast expectation-maximization microbial source tracking (FEAST). The potential contribution of cockroach bacteria to the floor dust-borne microbiome and indoor airborne microbiome was estimated to be 5.6% and 1.3%, respectively. Similarly, the potential contribution of cockroach PPB to the floor dust-borne microbiome and indoor airborne microbiome was calculated to be 4.0% and 1.2%, respectively. In residences with cockroach infestations, the contribution of other sources to the indoor environment was slightly increased. Collectively, the role of cockroaches in the transmission of microorganisms, particularly pathogenic bacteria and antibiotic resistance genes, cannot be overlooked.



KEYWORDS: *Cockroaches, Indoor environment, Microbiome, FEAST, ARG*

1. INTRODUCTION

In the context of rapid global urbanization, people spend the vast majority of their time indoors (>90%).[1] The indoor environment is closely related to human health.[2] Being primitive house-infesting insects, cockroaches are a serious public health concern to humans.[3,4] Globally, cockroaches continually invade human habitats, especially in low-income areas, where sanitation conditions are often poor.[3,5−8] The widely known health risk associated with cockroaches is from allergens.[9−11] The cockroach allergen has been identified as a strong asthma risk factor.[11] Recently, the role of cockroaches in carrying and spreading human pathogens to food or other surfaces has been reported.[12,13] Cockroaches carry pathogenic bacteria, increasing the risk of exposure to the occupants. Microorganisms carried by cockroaches may also act as adjuvants to enhance inflammation, thereby contributing to the development of allergies.[14,15] Moreover, cockroaches are carriers of antibiotic resistance genes (ARGs), which likely adds to the global concern on the ARGs, especially in indoor environments such as hospitals and residences.[16−18] Given the role of the microbiome in human health and disease,[19−22] the impact of the cockroach microbiome on household microbiome and human microbial exposome warrants attention.

Cockroaches are endosymbiotic insects that have coevolved with microorganisms over 300 million years of evolution.[23,24] For example, the commensal *Blattabacterium* facilitates nitrogen recycling from other waste.[25] Several potentially pathogenic bacteria are also frequently found in cockroaches, including *Enterobacteriaceae*, *Staphylococcus*, and *Mycobacte-*

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rium.[13,17,26] The cockroach gut microbiome is relatively stable, indicating a determinative effect of gut environments.[27] An overlap of 80.0% and 90.0% was found between the gut and fecal microbiome, suggesting that the cockroach gut microbiome could be disseminated via feces. However, distinct microbial compositions have been observed in laboratory- and field-collected cockroaches, with the latter showing more variability,[28] indicating that external factors might shape the cockroach microbiome. In a Libyan study, bacteria from cockroaches in hospitals harbored more antibiotic resistance than those in residences, providing evidence for the role of the environment in shaping cockroach microbiomes.[26] Because cockroaches can crawl freely everywhere, it is important to identify the influence of environmental factors on the cockroach microbiome.

Cockroaches colive with humans in indoor environments.[3,7] Human health is closely associated with the microbiome.[29−32] As people spend more than 90.0% of their time in indoor environments, the role of indoor microbiome exposure in shaping the human microbiome has become increasingly important.[19,33,34] Therefore, any factors that shape the indoor microbiome should not be ignored. As a "source" for indoor microbiome exposure, humans significantly contribute to the indoor microbiome via breathing, talking, sneezing, and other activities.[35−38] Despite the abundant microbiome carried by cockroaches,[23] there is little evidence regarding the role of cockroaches in spreading their microbiome. The role of cockroaches as a vector for spreading allergens and endotoxins derived from Gram-negative bacteria has been well characterized.[39,40] Residential cockroach samples have been reported to carry a wide variety of human pathogenic antibiotic-resistant bacteria, including *Staphylococcus aureus*, *Enterococcus* species, and *Klebsiella pneumoniae*.[16,18,41] By analyzing the clonal relationships between the pathogens from the cockroaches and the residents from the same residence, household cockroaches and human residents were found to share similar antibiotic-resistance determinants.[17]

Nevertheless, the quantitative relationships between household cockroaches and the associated indoor environmental microbiome and human microbiome are still unclear. Based on the knowledge of cockroach allergens and the vector role of cockroaches in carrying microorganisms, we collected cockroaches and parallel indoor environmental and human samples in residences across five cities in China. Then source analysis was performed to evaluate the microbial associations between cockroaches and the household microbiome. The results provide the first source analysis data on the microbiota relationships among cockroaches, humans, and the indoor environment, providing scientific data to fill research gaps in this area.

2. MATERIALS AND METHODS

2.1. Sample Collection

According to the geographical locations and climatic zones, five cities were selected, including Beijing (northern, temperate monsoon climate), Hangzhou (eastern, subtropical monsoon climate), Chengdu (southwestern, subtropical humid monsoon climate), and Fuzhou (southeastern, subtropical maritime monsoon climate), Guangzhou (southern, subtropical monsoon climate) (Figure S1). The presence or absence of a cockroach infestation in homes was used as a screening criterion. In each city, five residences infested with cockroaches and five cockroach-free residences were sampled for a total of 50 residences. Specifically, sampling in Beijing and Guangzhou was conducted in the summer season of 2019, and sampling for Chengdu, Fuzhou and Hangzhou was conducted in the summer of 2020. For each city, the cockroach boxes were sent to the volunteer residences in advance for 1 week's sampling. One week later, our sampling staff went to the city to harvest the cockroach samples and other types of samples. Due to the labor limitation, samples for different cities were collected sequentially. Temperature (T) and relative humidity (RH) in the residences of Beijing and Guangzhou were collected from the literatures (Beijing: T: 20.0−28.0 °C, RH: 40.0%−60.0%), Guangzhou (T: 21.5−32.0 °C, RH: 60.0%−100.0%).[42−44] For the other three cities, the temperature and RH information were recorded simultaneously during the sampling (Hangzhou (T: 27.0−34.2 °C, RH: 51.1%−69.2%), Chengdu (T: 23.4−30.2 °C, RH: 51.8%−85.2%), and Fuzhou (T: 29.1−33.5 °C, RH: 47.4%−68.4%)).

Cockroach samples were collected from infested residences, as reported by the residents. Before sample collection, sticky traps (Speedtox, Japan) were distributed to all enrolled residents. The traps were set at different locations (including bedroom, living room, and kitchen) within the house for 1 week. After 1 week, three cockroaches were randomly selected from each cockroach trap in every household as experimental subjects. The surface of the cockroaches was not treated and they were directly transferred into 50-ml sterile tubes containing 10-ml of sterile deionized water.[12,45,46] Then these cockroach suspensions were vortexed for 10 min and ultrasonicated in ice−water for 2 min. During the vortexing process, due to the occasional breaking of some cockroach samples, microorganisms from both the internal and external surfaces were washed off and considered to be part of the cockroach microbiome. Subsequently, the suspensions were centrifuged for 10 min (4 °C and 3000 g). The supernatant was used for later DNA isolation.

Indoor environmental and human resident samples were collected using procedures described elsewhere at the same time as the cockroach samples were harvested.[38] Indoor air samples (30.0 m^3) were collected in the living room (15.0 m^3) and bedroom (15.0 m^3) at a height of 1.3 m using a high-volume impactor (1.0 m^3/min, BioCTech, CAP1000, Beijing, China).[47] Floor dust samples were collected by swabbing floor areas (two, 5.0 × 10 cm^2) adjacent to the sticky trap box with a sterile flocking swab. Human samples included exhaled breath condensate (EBC) and skin samples. One resident from each residence participated in the sample collection. Enrolled participants were provided written informed consent (approved by the Biological and Medical Ethics Committee at Beihang University No: BM20180042). Briefly, EBC was collected with a portable collection device (developed by Peking University, Beijing, China) with the participant breathing continuously at a normal rate toward the device via a sterile tube for 6 min. Skin samples were collected by swabbing the forearm (5.0 × 10 cm^2); swabs were stored in tubes with 1.0 mL of sterile PBS. In total, 200 environmental and human resident samples were obtained. Unsampled swabs, mineral oil, and cockroach sticky traps were used as blank controls for the experiment.

For genetic analysis, samples of the same type collected in one city were pooled. In total, we analyzed 5 cockroach samples, 10 household indoor air samples (5 samples each for infested and control homes), 10 floor dust samples, 10 skin samples, and 10 EBC samples.

2.2. Bacterial Composition and Potential Pathogenic Bacteria

Bacterial diversity was assessed by 16S rDNA sequencing. DNA was extracted using the ALFA-SEQ advanced water DNA kit (Guangdong Magigene Biotechnology Co., Ltd., Guangzhou, China). DNA concentration and purity were assessed spectrophotometrically (NanoDropOne, Thermo Scientific, USA). The bacterial V3−V4 hypervariable region was amplified using specific primers (338F: 5′-ACTCCTACGGGAGGCAGCA-3′; 806R: 5′-GGACTACHVGGGTWTCTAAT-3′) on a GeneAmp 9700 PCR instrument (ABI, USA). The DNA library was prepared and sequenced on an Illumina Hiseq2500 platform (Guangdong Magigene Biotechnology, Guangzhou, China). The OTU table was obtained with a similarity level of ≥97.0% after processing the reads by various approaches. The OTU

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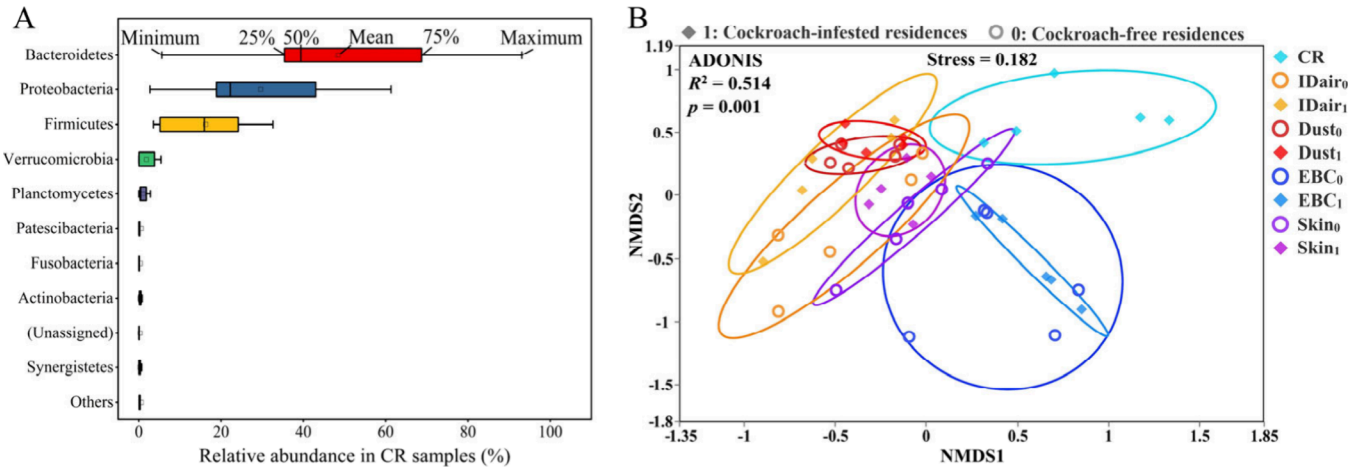

Figure 1. Characterization of bacterial community structures in cockroaches (CR), indoor environmental (IDair: indoor air; Dust: floor dust), and human resident samples (EBC: exhaled breath condensate; Skin: forearm surface). (A) Bacterial community structure at the phylum level in cockroaches from different cities. (B) NMDS plot visualizing the Bray−Curtis distance-based beta-diversity across samples of different types. Each data point represents one combined sample of a certain type from a certain city.

data for all samples were rarefied based on the minimal OTU count to bring all samples to the same scale for comparison. For subsequent analysis and comparison, the 16S data were subjected to rarefaction (Figure S2). The rarefied OTU table was used for diversity analysis and source apportionment. Additionally, the impact of different clustering methods on species annotation. We also used the QIIME2 software (v2022.8) with the DADA2 pipeline algorithm to generate the amplicon sequence variant (ASV) table.[48] Detailed data processing procedures have been described elsewhere.[49] Bacterial high-throughput sequencing data for all samples were uploaded to the National Center for Biotechnology Information under the accession number PRJNA955147.

Potential pathogenic bacteria (PPB) were screened against the List of Human Transmissible Pathogenic Microorganisms published by the National Health Commission of the People's Republic of China (http://www.nhc.gov.cn/wjw/gfxwj/201304/64601962954745c1929e814462d0746c.shtml) and the Majorbio Human Pathogens Database (https://microbiome.majorbio.com/pathogen). Following the approaches used in the previous studies, the pathogenic bacteria screening was conducted by comparing the taxonomic information at the genus level to the aforementioned database.[50−52]

2.3. Antibiotic-Resistance Gene Profiling

Quantitative PCR (qPCR) was used to measure the expression of 39 representative antibiotic-resistance gene (ARG) subtypes related to 7 frequently used antibiotics (tetracyclines, macrolides, β-lactams, vancomycins, sulfonamides, quinolones, and aminoglycosides). Two common MGEs (*intI 1* and *tnpA*) were screened for quantitative analysis. Reactions were performed with a StepOnePlu Real-Time PCR System (Thermofisher, USA). Primer sequences are listed in Table S1.[53] The relative abundance of each ARG was evaluated the $2^{-\Delta\Delta Ct}$ ($\Delta Ct = CT_{(ARG)} - CT_{(16s\ rRNA)}$) method, where *CT* is the threshold cycle.[53,54]

2.4. Statistical Analysis

Independent *t* tests were performed to evaluate differences between groups with a *p* value of 0.05 (SPSS 27.0, USA) after the normality test using the Shapiro−Wilk test ($p = 0.05$). The Kolmogorov−Smirnov nonparametric test was used to compare significant differences between two independent groups of samples. Nonmetric multidimensional scaling (NMDS) analysis based on Bray−Curtis distance was used to visualize the relationships between bacterial communities using R (4.1.3) (vegan, ggplot2, and ape). Permutational multivariate analysis of variance (ADONIS) was used to compare two different groups of samples ($p = 0.05$) (R "vegan"). Linear discriminant analysis effect size (LEfSe) analysis was performed to identify indicator bacterial taxa and phylogenetic dendrogram visualization was obtained using OmicStudio (https://www.omicstudio.cn/tool/). Functional prediction of the bacterial communities was performed using PICRUSt2 (http://cloud.magigene.com/). Spearman rank correlation (r) of bacteria with ARGs was calculated using SPSS 27.0. Network analysis was conducted based on the significant positive correlations between bacteria and ARGs ($r > 0.6$, $p < 0.05$). Network visualization was performed using the Gephi platform (V 0.10).

Fast expectation-maximization source tracking framework (FEAST) was used to investigate the microbial associations between cockroaches, environment, and humans using 16S rDNA data.[38] Similar community structures were obtained while using the OTU clustering method and amplicon sequence variants (ASVs) method (Figure S3A). However, high level of unclassified taxa was seen while using amplicon sequence variants (ASVs) clustering method (Figure S3B). This phenomenon has been observed in previous literatures, which likely affects the FEAST source apportionment results.[55,56] Therefore, the associations between the CR microbiome and indoor environmental, human microbiome were estimated with the OTU-based taxonomic data. The potential contribution of environmental and human samples to cockroaches was evaluated by assuming the cockroach was a sink and others as sources. Vice versa, the impact of the cockroach microbiome on the environmental and human microbiomes was evaluated by assuming the cockroach microbiome was a source.

3. RESULTS AND DISCUSSION

3.1. Bacterial Community in Cockroaches and the Association with Indoor Environmental and Human Resident Microbiome

Diverse bacteria were found in cockroaches collected from dwellings across the 5 cities. This diversity was reflected in the alpha indices, which were comparable to those from environmental and human bacterial samples (Figure S4). At the phylum level, cockroaches carried predominantly Bacteroidetes (median: 39.4%; range: 5.6%−93.1%), followed by Proteobacteria (median: 22.3%; range: 2.7%−61.3%) and Firmicutes (median: 15.9%; range: 3.6%−32.7%) (Figure 1A). Similar phylum structures have been observed in both lab- and field-collected cockroaches, with Bacteroidetes being predominant.[28]

The genus *Blattabacterium*, of phylum Bacteroidetes, dominated at the genus level (median: 34.5%; range: 5.4%−

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91.3%). As an endosymbiont, *Blattabacterium* facilitates nitrogen acquisition and is mainly found in the cockroach gut or feces.[25,28] Proportions of other genera, such as *Acinetobacter, Bacillus*, *Pseudomonas*, *Streptococcus*, and *Staphylococcus*, were generally low. These lower-level genera are likely from the environment surrounding the insects.[57]

Large variations were observed in the bacterial communities of cockroaches from different locations (Figure 2). The dominant *Blattabacterium* accounted for >90.0% of the Fuzhou (FZ) cockroach samples but <10.0% of cockroaches from Hangzhou (HZ). For HZ cockroaches, the proportions of *Acinetobacter* (31.1%) and *Bacillus* (23.2%) were higher than those in cockroaches from other cities. This variation could be associated with several factors; for instance, sex of cockroaches might play a role in the different ratios of *Blattabacterium*, with a higher ratio in males (>63.4%) than in females (<7.8%).[27] In our study, cockroach samples obtained from five residences in each city were pooled for high-throughput sequencing analysis and sex was not differentiated while pooling. It is also possible that regional environmental differences played a role. As a source for the environmental microbiome, bacterial structure similarities have been observed between the cockroach and their surrounding environment.[12,58] For instance, cockroaches in hospitals have been found to carry high levels of hospital-associated drug-resistant pathogens.[57] Besides, previous studies have reported the high abundance of *Bacillus* in the air of Hangzhou, which is consistent with the higher load of *Bacillus* in cockroaches from Hangzhou.[59−63] This situation may be caused by differences in the indoor environments of the various sampling locations. For example, *Bacillus* has been found to prefer growing in warm and humid indoor environments (high temperature and high relative humidity).[64,65] This study also found that cockroach samples from areas with elevated indoor temperatures and humidity (Hangzhou: 23.21%; Guangzhou: 1.02%) contained higher levels of *Bacillus* (Figure 2). Regional differences have also been observed in the bacteria carried by cockroaches from different cities.[57]

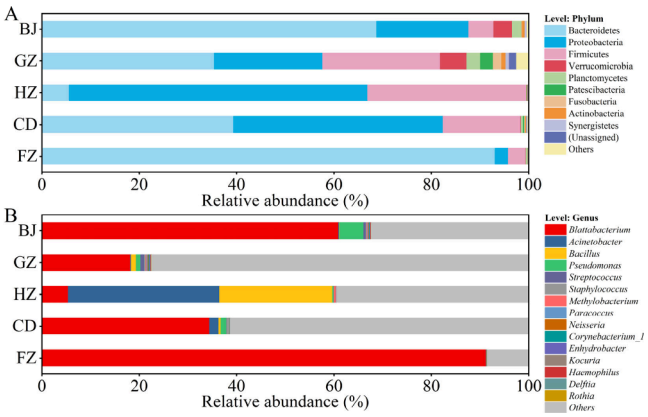

Figure 2. Relative abundance of bacteria in cockroaches from five different cities at the level of (A) phylum and (B) genus. BJ: Beijing; GZ: Guangzhou; HZ: Hangzhou; CD: Chengdu; FZ: Fuzhou.

We compared the bacterial communities in cockroaches to those in the residential environment and human residents. Despite the high varieties in various types of cockroach samples, bacterial communities showed significant differences compared to the other samples (Figures 1B and S5, and Table S2). This is further evidence that cockroaches have a diverse and relatively stable microbiome and that the factors influencing the microbial composition of the cockroach body surface are complex.[27] *Blattabacterium* accounted for a low ratio in the environmental and human samples (median: 0.11%; range: 0.01%−0.68%). On the other hand, *Acinetobacter*, *Kocuria*, *Streptococcus*, and *Delftia* were the dominant genera in IDair (45.1% ± 40.6%), Dust (14.0% ± 9.1%), EBC (26.1% ± 13.3%), and Skin (17.7% ± 20.0%), respectively.

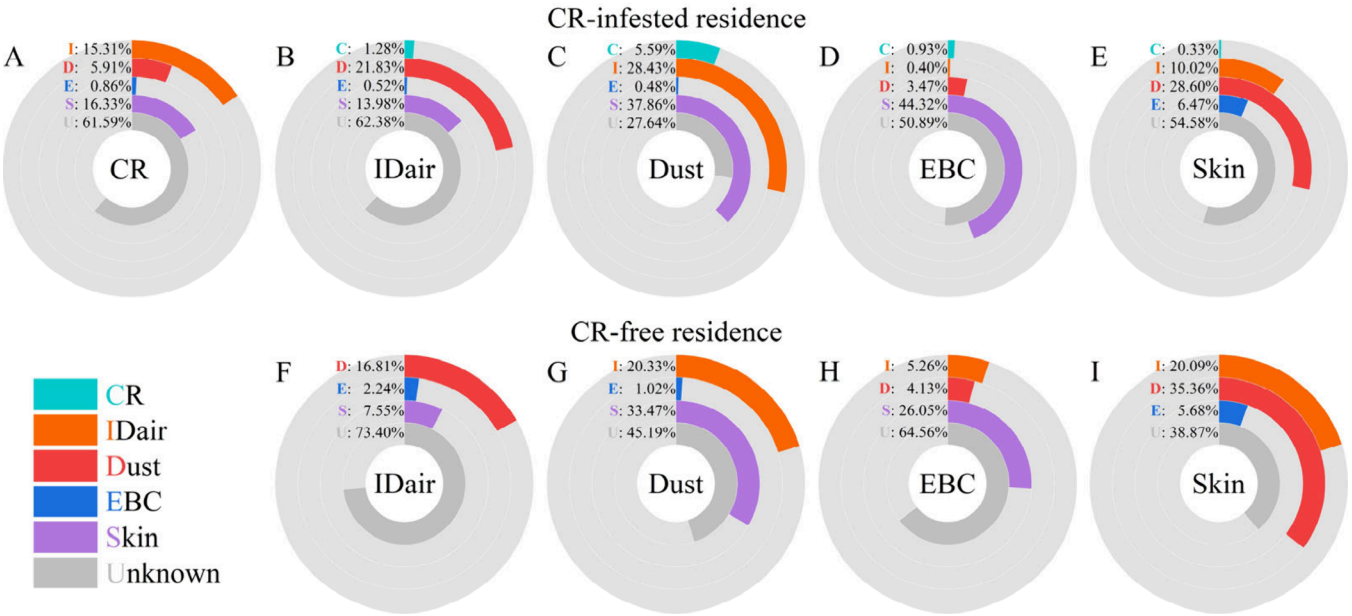

Figure 3. Potential contribution of bacteria from various source samples to the sink: (A) Cockroach (CR), (B) indoor air (IDair), (C) floor dust (Dust), (D) resident exhaled breath (EBC), (E) resident forearm (Skin) at cockroach (CR)-infested residences, and (F) IDair, (G) Dust, (H) EBC, and (I) Skin at cockroach (CR)-free residences. The text in the center of the concentric circles indicates the type of sink. Each number represents the average across the five cities.

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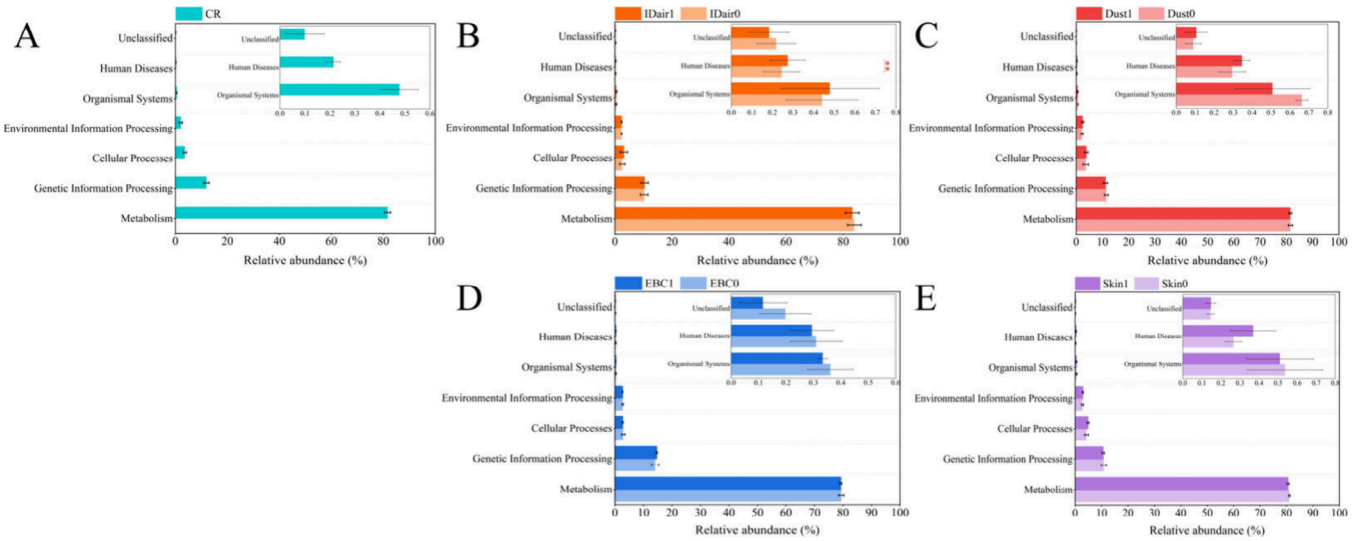

Figure 4. KEGG functions of bacteria in cockroaches (A: CR), indoor environmental samples (B: IDair, C: Dust), and human resident samples (D: EBC, E: Skin). Subscripted number "0": samples from cockroach-free residences, "1": samples from cockroach-infested residences.

However, the relative abundance of dominant genera in these samples was low in cockroach samples (*Acinetobacter*: 6.7%, *Kocuria*: 0.05%, *Streptococcus*: 0.21%, and *Delftia*: 0.03%).

The potential origins of cockroach bacterial compositions were investigated by assuming the cockroach as the sink and EBC, skin, indoor air, and floor dust as possible sources using FEAST analysis (Figure 3A, Table S3). The potential contribution from each sample type to the cockroach bacterial community was skin (mean: 16.3%; median: 1.3%), indoor air (15.3%; 3.8%), floor dust (5.9%; 3.6%), EBC (0.86%; 0.06%), and unknown (61.6%; 77.7%). The mean contribution of four external microbiome sources thus accounted for more than one-third of the cockroach microbiome. Although cockroaches do not appear to be in direct contact with human skin, our results confirm that the bacterial community of skin samples from residents had the strongest relationship with the bacterial community of cockroaches compared to the other three groups of indoor samples. This is likely associated with the fast incorporation of occupant's skin debris into the indoor environmental microbiome and the subsequent impact on the cockroach microbiome.[38,66−69] In short, the results here indicate that the cockroach microbiome is a confluence of its internal resident and external environmental microbiomes.

The potential impacts of cockroach bacteria on the indoor environmental microbiome were evaluated by assuming cockroaches as a source and the environmental microbiome as a sink (Figure 3, Tables S4 and S5). Cockroach bacterial communities likely contributed an average of 1.3% (median: 0.44%; range: 0.10%−5.2%) to the indoor air microbiome and 5.6% (median: 0.55%; range: 0.19%−25.9%) to the floor dust microbiome. Cockroaches contributed a slightly higher percentage of floor dust bacteria than the indoor airborne microbiome. This result is reasonable, given that the insects crawl on the floor. However, no significant difference was observed between the samples from cockroach-infested and cockroach-free residences (K−S test, $p > 0.05$, Table S6).

In households with cockroach infestations, the potential contribution of the human microbiome (EBC + skin) to the microbiome spread in indoor airborne (14.5%) and floor-dustborne (38.3%) was slightly higher than in households without cockroaches (indoor airborne: 9.8%, floor-dustborne: 34.5%; K−S test, $p > 0.05$). This is an interesting phenomenon, suggesting that in households with cockroaches, the exchange of microorganisms between residents and the indoor environment is more frequent.

The presence of cockroaches reflected indoor hygiene conditions, to some extent. Compared to the cockroach-infested residences, the unknown sources for the indoor airborne and dust-borne bacteria were slightly higher in cockroach-free residences, likely due to differences in hygiene (K−S test, $p > 0.05$). This supports the hypothesis that worse hygiene conditions in cockroach-infested residences enhanced the microbiome contribution from both human and animal residents to the indoor microbiome, thus indicating a low-quality indoor environment from the microbiological perspective.[70] Thus, bacteria from indoor sources, for instance, cockroaches and human residents, exert a strong effect on the indoor microbiome. In cockroach-free residences, the unknown sources could originate outdoors; however, outdoor air samples were not evaluated in this study.

Poor hygiene conditions in cockroach-infested residences were also reflected in the amount of bacterial indicator taxa. The indicator taxa for each type of samples in cockroach-free and cockroach-infested residences were investigated using LEfSe analysis (Figures S6 and S7), which showed that the number of indicator taxa for samples collected in cockroach-infested residences was greater than in cockroach-free residences. This supports the hypothesis that poor hygiene conditions in cockroach-infested residences enhanced the microbiome contribution from both human and animal residents to the indoor microbiome, thus indicating a poor-quality indoor environment from a microbiological perspective.

An association was also observed between the cockroach bacterial communities and human expiratory bacteria (EBC), suggesting an inhalation risk for human residents (Figure 3D, Table S7). Additionally, gene function prediction analysis showed that genes related to human diseases (infectious disease: parasitic, neurodegenerative disease, and cancer: overview) were slightly higher in the indoor air and floor dust samples at cockroach-infested residences than at cockroach-free residences (Figure 4). The causal relationship between cockroach allergen and exacerbation of asthmatic

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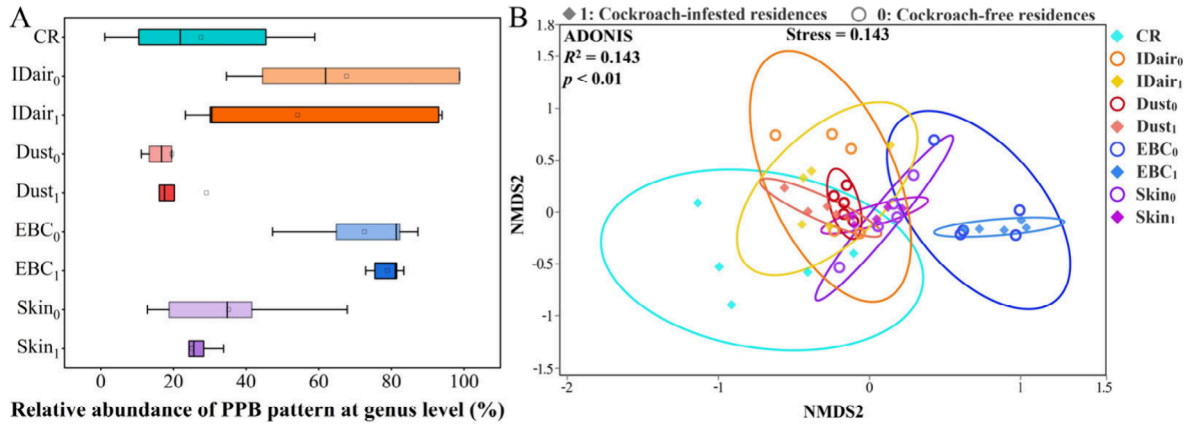

Figure 5. Potential pathogenic bacteria (PPB) patterns in cockroaches (CR), indoor environment (IDair: indoor air, Dust: floor dust), and human resident samples (EBC: exhaled breath condensate, Skin: forearm surface). (A) Relative abundance of PPB in total bacteria at the genus level. (B) NMDS plot visualizing the PPB beta-diversity based on the Bray−Curtis distances. Each data point represents one combined sample of a certain type from a certain city.

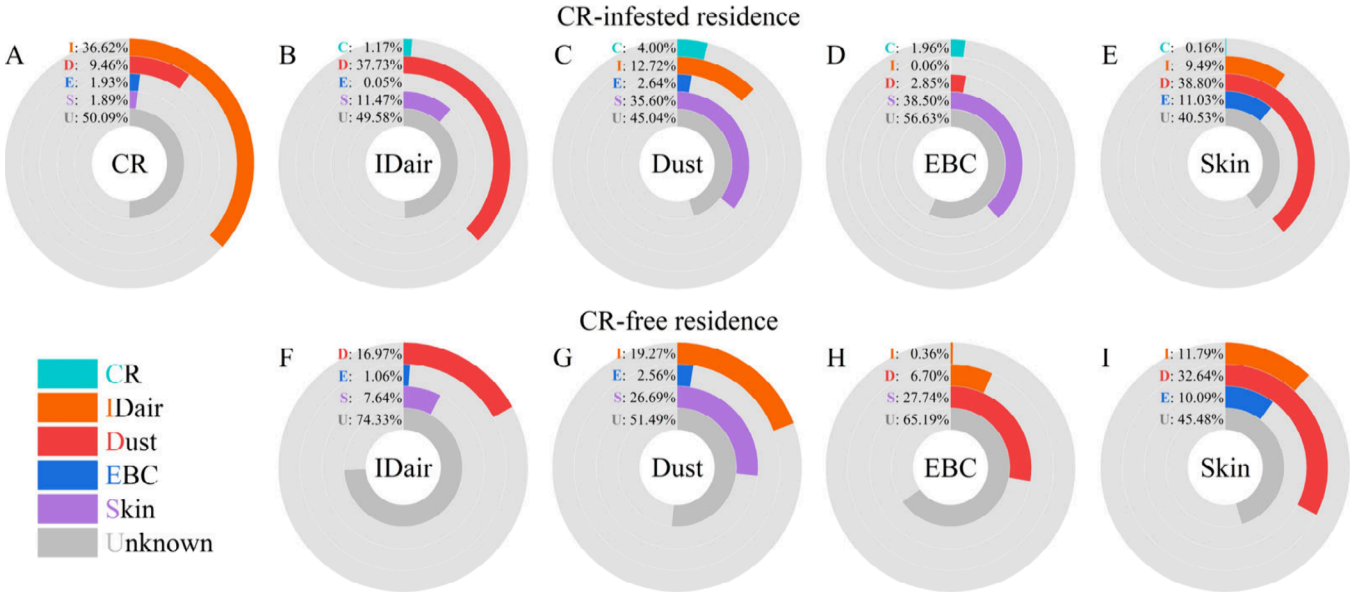

Figure 6. Potential contribution of potential pathogenic bacteria (PPB) from source to sink samples: (A) Cockroaches (CR), (B) indoor air (IDair), (C) floor dust (Dust), (D) human resident exhaled breath (EBC), (E) resident forearm (Skin) at cockroach-infested residences, and (F) IDair, (G) Dust, (H) EBC, and (I) Skin at cockroach-free residences. The text in the center of the concentric circles indicates the type of sink. Each number represents the average across the five cities.

individuals sensitive to cockroaches is sufficient;[71,72] however, no sufficient evidence is available for asthmatic individuals not sensitized to cockroaches.[73] Cockroaches have also been implicated as a source of indoor airborne endotoxins.[39] Therefore, there is a possibility that bacteria or bacterial components from cockroaches serve as a trigger or irritant for individuals with asthma not sensitized to cockroaches.[74,75] Inhalation exposure to cockroach allergens should be explored by considering microbiome exposure.

3.2. Potential Pathogenic Bacteria (PPB) in Cockroaches and Their Associations with Those in the Indoor Environmental and Human Resident Microbiome

The percentage of PPB in cockroaches varied significantly, ranging from 1.1% to 58.9% (Figure 5, Table S8). The PPB patterns in cockroaches from different cities also varied significantly (Figures 5 and S8). Moreover, the cockroach PPB pattern differed from those in human residents, indoor air, and floor dust (Figure S8). This is consistent with the results of the comparison between the total bacterial community structures of cockroaches and other external microbiomes and indicates that, except for their internal microbiome, the external environment also significantly affects the PPB carried by cockroaches.

The impact of external environmental PPB on the cockroach PPB was assessed by using FEAST analysis (Figure 6A). The mean potential sources of cockroach PPB include indoor air (36.6%), floor dust (9.5%), and EBC (1.9%). Specifically, the contribution from the indoor air samples ranged between 23.3% and 98.8%. Due to the fact that cockroaches could crawl through various environments, the role of cockroaches in transporting PPB should not be ignored.

The abundant PPB in cockroaches includes *Enterococcus*, *Acinetobacter*, *Stenotrophomonas*, *Pseudomonas*, *Bacteroides*, *Bacillus*, *Alistipes*, *Lactobacillus*, and *Streptococcus* (Figure S9). Several of the PPB genera were also common in other types of

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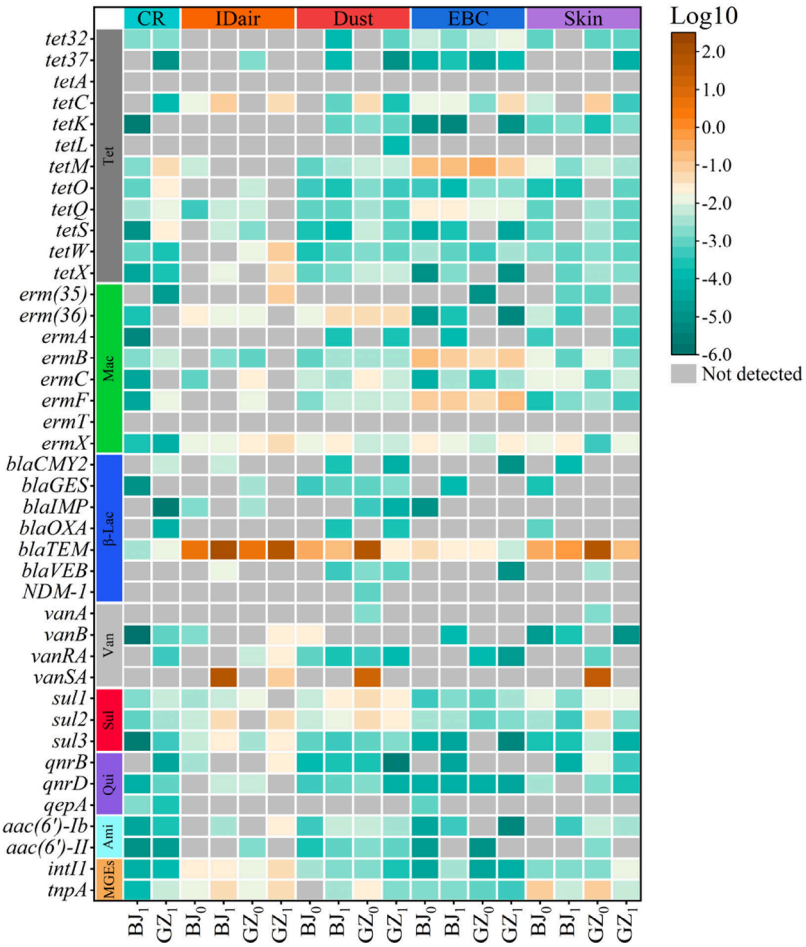

Figure 7. Relative abundance of the seven types of ARGs and MGE subtypes in cockroaches (CR), indoor environment (IDair: indoor air, Dust: floor dust), and human resident samples (EBC: exhaled breath condensate, Skin: forearm surface) using $2^{-\Delta\Delta CT}$ values. BJ: Beijing; GZ: Guangzhou. Subscripted number "1": samples from cockroach-infested residences, "0": samples from cockroach-free residences.

samples. For instance, *Streptococcus* was common in EBC samples, and *Acinetobacter* was abundant in skin, indoor air, and dust samples.

Acinetobacter baumannii is the most common pathogen associated with both community-acquired and nosocomial pneumonia.[76,77] The percentage of *Acinetobacter* in cockroaches from Hangzhou was the highest (31.1%), accompanied by a relatively high ratio in human and environmental samples (skin: 5.3%, air: 91.8%, and dust: 3.9%). The high prevalence of *Acinetobacter* in cockroaches and indoor air indicates a need to pay attention to the present environmental PPB. *Streptococcus pneumoniae* (tuberculosis) and group A *streptococci* (strep throat) are the two most common pathogens. Around one-quarter of people globally is infected with *S. pneumoniae*.[78] Therefore, the role of cockroaches in carrying and spreading *Streptococcus* should be considered.

The potential effect of cockroach PPB on the environmental and human resident microbiome was evaluated by assuming the cockroach PPB as a source (Figure 6B–E). The cockroach PPB contributed, on average, 1.2% to indoor air, 4.0% to floor dust, and 2.0% to the EBC microbiome. These data suggest that the impact of PPB on the microbiological quality of the indoor environment and human health cannot be ignored. A meta-analysis indicated that bacteria carried on the exterior of cockroaches pose a greater threat than those within their bodies.[57] Numerous studies have shown that a large proportion of the pathogens carried by cockroaches are drug-resistant, and some are even multidrug resistant.[13,41,57] Since cockroaches are barrier-free and can crawl anywhere, it is important to pay more attention to the PPBs carried by cockroaches.

For cockroach-free residences, the potential contribution percentages from the investigated sources to each type of sink sample were lower than those at the cockroach-infested residences (Figure 6F–I). Specifically, the potential contributions from investigated known sources were 25.7% for the indoor air as the sink, 38.5% for the floor dust, and 34.8% for the sink EBC, 54.5% for the skin as the sink. These values were all lower than those at the cockroach-infested residences, which were 50.4% for the sink indoor air, 55.0% for the sink floor dust, 43.4% for the sink EBC and 59.5% for the sink skin (K–S test, $p > 0.05$). It is therefore possible that the appearance of cockroaches enhances the microbial interaction between the indoor environment, cockroaches, and human residents. These comparisons further strengthened the importance of human household hygiene. It is recommended that occupants pay more attention to household hygiene and take measures such as timely removal of garbage in the kitchen and frequent indoor ventilation.

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3.3. ARG Profile in Cockroaches and Associations with Indoor Environmental and Human Resident Microbiomes

Diversified ARGs were overserved in cockroaches. Among the 39 measured ARGs, the number of ARGs in cockroaches was 24 (Beijing) and 27 (Guangzhou) (Figure S10). The detected ARG numbers were no less than those in the human skin microbiome (22, 19, 25, and 24) and in the EBC samples (24, 25, 18, and 24) at four types of residences. The abundance of ARGs in cockroaches is consistent with those found in laboratory cockroach controls and those treated with antibiotics.[23] This is because both cockroaches and humans live in symbiosis with the microbiome, and cockroaches are also considered potential hosts for the spread of ARGs.[79] The coexistence of ARGs and mobile genetic elements (MGEs) indicates that cockroaches could serve as a reservoir and transmission vector.[12,23] Due to their ubiquitous nature and diverse food sources, they are also controlled by various insecticides, and the role of cockroaches in residential ARG spread should not be ignored.

A significant difference was found between the ARG profiles of cockroaches from Beijing and Guangzhou ($p < 0.05$) (Figure 7). Generally, ARGs against tetracycline were predominant in cockroaches from both cities, followed by macrolides and beta-lactams. The dominance of ARGs against tetracycline differed from that found in laboratory-reared cockroaches, which predominantly carry beta-lactam ARG.[23] Treatment with antibiotics increased the frequency of ARGs in cockroaches.[23,80] Therefore, the difference between residence- and lab-raised cockroaches is likely related to environmental factors.

Similarly, in indoor samples from both cities, the ARG profiles predominantly exhibit resistance to β-lactams (*blaTEM*, *blaCMY2*, *blaVEB* and *blaCES*) and macrolides (*ermB*, *ermC*, *erm(35)* and *erm(36)*). This is because beta-lactam and macrolide antibiotics have been used extensively in human and veterinary drugs.[81] This similar pattern of antibiotic resistance also suggests that cockroaches may be an important sink for ARGs in the indoor environment, somewhat accelerating the spread of ARGs in the indoor environment. In contrast to the cockroach samples, higher levels of sulfonamide ARGs (*sul1*, *sul2* and *sul3*) were found in some indoor samples (IDair, Dust and Skin). The prevalence of sulfonamide ARGs in indoor environments has also been found in several previous studies.[82−84]

Two common MGEs (*intI1* and *tnpA*) were detected in all cockroach samples, with relative abundances ranging from 1.9×10^{-4} to 8.3×10^{-3} (Figure 7). Notably, the relative abundance of MGEs in the cockroach samples was relatively low. Specifically, the levels of *intI1* and *tnpA* in cockroaches were similar to those in the EBC samples but one or 2 orders of magnitude lower than those in the skin, indoor air, and floor dust samples. These low levels of MGEs in living organisms and high levels in the indoor environmental samples were likely the result of environmental selective pressure. A similar phenomenon was reported in human, animal, and ambient environment samples.[85]

At cockroach-infested residences, the indoor airborne ARG compositions were more diverse and abundant than those in cockroach-free residences. The abundance of ARGs detected in indoor air in this study was higher than previously observed in Hong Kong and Beijing.[82,86] This is likely related to differences in sampling methods. Specifically, air conditioning filter dust and indoor dust were used in prior studies. Thus, household air is an important reservoir and vector of ARGs.

Moreover, the relative abundance of indoor airborne MGEs at cockroach-infested residences was also slightly higher than that at residences without cockroaches (for intl1: $IDair_1$ (3.3×10^{-2}), $IDair_0$ (2.2×10^{-2}), indicating a high risk of horizontal transfer for airborne bacteria.[87,88]

The potential bacterial ARG hosts were identified using the network analysis based on the Spearman rank correlation coefficient with a threshold value of 0.6 ($p < 0.05$) (Figure S11).[89−94] Notably, the relatively high abundance of PPBs in cockroaches from Beijing was significantly associated with ARGs, i.e., *Streptococcus* (*tetM*), *Acinetobacter* (*blaTEM*, *sul3*, and *erm(36)*), and *Staphylococcus* (*sul3*, *blaTEM*, *blaCMY2* and *tnpA*). Different associations were found for cockroaches in Guangzhou, i.e., *Enterococcus* (*vanB*), *Acinetobacter* (*intI1*) *Pseudomonas* (*tnpA*, *sul3*, *vanRA* and *qnrB*) and *Alistipes* (*vanB*). Considering the role of MGEs in the horizontal transfer capacity of ARGs,[95] a stronger association between certain PPBs and MEGs indicates a possibility of ARGs transfer between bacteria carried by cockroaches and other samples.[96] The different host-ARG associations in Beijing and Guangzhou indicate the possibility that environmental factors influence the bacterial compositions and antibiotic resistance in cockroaches. The finding of multidrug resistant bacteria and vancomycin-resistant *Enterococcus* are all serious concerns for human residents.[97−99] Zoonotic transmission of ARGs is an emerging concern within households,[100] and, given the flexibility and ubiquity of cockroaches, the zoonotic transmission of ARGs related to cockroaches in human dwellings should be taken seriously. Maintaining a clean living environment is necessary to inhibit cockroach infestation and indirectly reduce the risk of exposure to ARGs for residents.

4. CONCLUSIONS

We profiled cockroach bacteria and their associations with the human resident and indoor microbiome, PPB, and ARGs by simultaneous sampling of cockroaches and human resident and indoor environmental samples at 5 homes across cities. Several limitations hinder further in-depth discussions from a health perspective. Cockroach types were not differentiated, although there may be differences between insects found in the north and south of China. Cockroaches and other types of samples collected from five residences per city were pooled together, respectively. Samples from the five cities were sequenced for bacterial taxonomy identification, and samples from two cities (Beijing and Guangzhou) were investigated for their ARGs characteristics. Therefore, there was no repetition for the samples that represent each city during the sequencing process, which might increase the uncertainty in the results. Based on cockroach samples, exhaled breath condensates, and skin swabs from human residents and indoor air and floor dust samples, the current work focused on the correlations between the microbiome and ARGs of these sample types. We did not collect information regarding cockroach allergen levels or resident sensitization. Future research should also take into account the impact of factors, such as the type of housing, the living habits of the occupants, and the economic income levels of the occupants. Health conditions and relevant human samples should be collected for the exposure-impact study.

Being a common household pest, cockroach allergens have been found in more than three-quarters of urban homes in the United States.[40] Accompanying the shedding of cockroach

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allergens, the cockroach microbiome is also released. The adverse health effects of cockroach allergens are clear; however, the effect of the cockroach microbiome is unclear. Various studies have investigated the cockroach microbiome, but few have investigated their associations with the human and indoor environmental microbiome. The results from the current study have provided data supporting the bidirectional interaction among the microbiomes of cockroaches, human residents, and the household environment.

Urbanization has fostered the exposure of city dwellers to cockroaches.[101] Human settlements infested with cockroaches are generally characterized by poor hygiene conditions. This study confirmed that cockroaches not only directly contributed to the microbiome but also indirectly enhanced the contributions from other sources, enabling them to exert a strong force in shaping the indoor microbiome. Considering the effects of the indoor microbiome on human health and widespread cockroach infestation in human dwellings, more efficient and eco- and human-friendly measures that do not create antibiotic- or pesticide-resistant problems should be taken to eliminate cockroaches in human household environments.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/envhealth.4c00216.

Location of sampling sites; microbial community characteristics of samples; number of subtypes of ARGs detected; network analysis of ARGs with bacteria; primer information for ARGs; results of source analysis for cockroaches and indoor samples (FEAST) (PDF)

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Notes

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