



Characterization of 16S rRNA Metagenomic Amplicon Data of Bacterial Communities Associated with IGR Treated Tropical Bed Bugs, *Cimex hemipterus* (F.) (Hemiptera)

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Highlights

- Metagenomic characterization of bacterial communities in insecticide-treated *Cimex hemipterus*, revealing Proteobacteria (>99%) as the dominant phylum across all samples.
- Wolbachia and Burkholderia-Caballeronia-Paraburkholderia identified as key endosymbionts, with pyriproxyfen treatment showing distinct shifts in their abundance compared to control samples.
- Insecticide exposure has minimal impact on overall gut bacterial diversity, but subtle changes in secondary symbionts suggest potential symbiont-mediated mechanisms influencing insecticide susceptibility.

EARLY VIEW

Characterization of 16S rRNA Metagenomic Amplicon Data of Bacterial Communities Associated with IGR Treated Tropical Bed Bugs, *Cimex hemipterus* (F.) (Hemiptera)

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Running head: Bacterial Communities in Treated Bedbugs

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Abstract: Endosymbiont serves crucial physiological functions within their insect hosts, including nutrition provision, metabolic enhancement, and detoxification stimulation. The exploration of the microbiome population makeup corresponding to tropical bed bugs (*Cimex hemipterus*) became feasible with the advancement of novel metagenomic approaches. This study examined the microbial composition of tropical bed bugs treated with four different classes of insecticides: chlorfluazuron, tebufenozide, pyriproxyfen, β -cyfluthrin + imidacloprid, and control. Next, MiSeq Illumina was used to sequence the hypervariable section (v3-v4) of the 16S rRNA gene region. Proteobacteria accounted for over 99% of the overall microbial population in all samples. The two most common OTUs at the genus level were *Wolbachia*, an alpha-proteobacterium, and *Pectobacterium*, a gamma-proteobacterium. Despite some similarities in the major OTUs of the bacteria, the genera represented in the lower abundances were quite diverse. The bacterial abundance of bed bugs treated with pyriproxyfen exhibited a notable disparity from the control sample. This research lays the groundwork for a previously unknown symbiont-mediated mechanism influencing insecticide susceptibility in bed bugs.

Keyword: Endosymbionts, *Cimex hemipterus*, metagenomic, Proteobacteria

Abstrak: Endosimbion memainkan peranan penting dalam fisiologi serangga perumah, termasuk biosintesis nutrien, modulasi metabolisme dan penyahtoksikan. Kemajuan teknologi metagenomik membolehkan pencirian terperinci komuniti mikrobiom, termasuk dalam pepijat tropika *Cimex hemipterus*. Kajian ini menilai struktur komuniti bakteria dalam *C. hemipterus* selepas pendedahan kepada empat kelas racun serangga: chlorfluazuron, tebufenozide, pyriproxyfen dan campuran β -cyfluthrin + imidacloprid, dan membandingkannya dengan kumpulan kawalan. Penjujukan kawasan hiperubah V3–V4 gen 16S rRNA menggunakan pendekatan kultur-bebas metagenomik dijalankan untuk mengenal pasti kepelbagaian taksonomi bakteria. Hasil menunjukkan filum Proteobacteria mendominasi (>99%) dalam semua sampel. Pada peringkat genus, *Wolbachia* dan *Burkholderia*–*Caballeronia*–*Paraburkholderia* merupakan taksa dominan merentas kumpulan rawatan dan kawalan. Indeks kepelbagaian alfa (Shannon, Simpson, Chao1) tidak menunjukkan perbezaan signifikan ($p > 0.05$). Walaupun demikian, beberapa OTU memperlihatkan perubahan kelimpahan relatif, terutama pada sampel yang dirawat dengan pyriproxyfen. Keseluruhannya, pendedahan kepada racun serangga tidak menjejaskan kepelbagaian bakteria secara menyeluruh, namun perubahan selektif pada endosimbion tertentu mencadangkan interaksi antara mikrobiom dan respons fisiologi terhadap tekanan kimia. Dapatan ini menyediakan asas untuk penyelidikan lanjut mengenai peranan simbiosis mikrob dalam mekanisme toleransi atau kerentanan serangga terhadap racun serangga.

Kata Kunci: Endosimbion, *Cimex hemipterus*, metagenomik, Proteobacteria

INTRODUCTION

Cimex is a well-known hematophagous species, with *Cimex lectularius* and *Cimex hemipterus* being the most closely related to humans. Bed bugs were initially described as the prey of bats, birds, and mammals, but they successfully adapted to humans who shared the same cave in the Mediterranean and Central Asia regions (Akhoundi et al., 2020). As urbanisation and civilization advance, humankind makes an effort to enhance their living conditions, and bed bugs adapt to this new way of life in tandem. As civilization and urbanization perks, humans begin to strive to improve their living conditions and bed bugs evolve alongside with them to the new live style. Similar to mosquitoes, a blood meal serves as the essential nutritional source required for the survival, development, and reproduction of this cimicid species (Akhoundi et al., 2020; Dieng et al. 2017). Hence, they skillfully locate their host by the emanation of body odor, heat and carbon dioxide from their sleeping hosts (Doggett et al.,

2012; Goddard & DeShazo, 2009). Bed bugs, belonging to the hemimetabolous metamorphosis insect order, progress through seven life stages where each stage needs a blood meal for development. The entire life cycle of bed bugs, from oviposition to pupation, typically spans between 30 and 70 days yet this duration is highly contingent upon environmental conditions and the availability of hosts (Harlan 2006; Delaunay et al. 2011; Okwa & Omoniyi 2016). Interestingly, this robust pest can survive months without bloodmeals beside their fast population advancement, making them difficult to combat. It was widely documented in history that mankind had successfully overcome the burden of this formidable ectoparasite yet recently they had spread over the world like a plague, especially in developed areas like Europe, North America, Australia, and Eastern Asia (Doggett et al., 2004; Potter, 2007). The present rise was prompted by numerous factors, including travel, global warming, recycling infested furniture, periodic usage of bait traps with chemical residue, and establishment of resistance across the bed bugs population (Benoit, 2007; Lim & Ab Majid 2021). The resistance mechanism plays a pivotal role in re-infestation issue where bed bug capable in modulating three type of resistance competence in which behavioral resistance, physiological resistance, and symbiont-mediated resistance. According to Rupawate et al. (2023), symbionts often fulfil the nutritional requirements of their hosts, support general growth, aid in reproduction, protect against pathogen infections, control host gene expression, and undertake biochemical detoxification of toxins and pesticides. This phenomenon allows bed bugs to persist and extend their life cycle in spite of being subjected to a range of treatment methods. Bedbugs are hosts to a diverse array of microbial symbionts, which exist in varying quantities. Among these, *Wolbachia* is widely acknowledged as one of the most ubiquitous microorganisms found in bedbugs, because it provides their host insect with nutrients such as vitamin B (Lim & Ab Majid 2021). 16S rRNA metagenomics analysis widely employed for exploration of microbiota of a sample host as this new generation sequence analyzes and amplifies the V3-V4 hypervariable region to identify the bacterial diversity (Bukin et al., 2019; Gupta et al., 2019). To further understand the impacts of insecticides on the bacterial population of *C. hemipterus*, samples were examined and characterized employing a 16S rRNA metagenomic culture-independent technique, subsequently followed by MiSeq Illumina sequencing.

MATERIAL AND METHOD

Bed Bug

Tropical bed bugs were collected from residential houses around Penang Island and mass reared in a laboratory setting to imitate their harborage to promote their longevity and

reproduction. Employing surface contact bioassay, 5 adults per replicate were treated with four classes of insecticides at four doses for 2 week interval. Then, the *C.hemipterus* were preserved in 95% ethanol for 16S rRNA metagenomic culture-independent analysis.

Microbial DNA Extraction

A single bed bugs from each replicate of bioassay treatment and control were selected and sterilized using 70% ethanol and then drown in distilled water for molecular analysis (Cappelli et al., 2022). HiYield Genomic DNA isolation kit (Real Biotech Corporation, Taiwan) used to separate microbial DNA from the bed bug's sample. The concentration and purity of the microbial DNA samples extracted were evaluated using a Quawell Micro Volume Spectrophotometer (Labgene Scientific, Switzerland) and visualized on agarose gels.

16S Illumina Library and Sequencing

Samples of microbial DNA that had been extracted were delivered to a service provider to undergo library assembly and 16S rRNA metagenomic identification. 20 microliters mixture of 5 FastPfu Buffer (4 μ l), 2.5 mM dNTPs (2 μ l), primer 5 μ M (0.8 μ l), FastPfu Polymerase (0.4 μ l), and template DNA (10 ng) were utilized. Polymerase chain reaction method was used to make copies of the bacteria's marker region. The procedure included 25 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. The primer sequence used was 341F-80R.

16S Metagenomic Data Analysis

The raw fastq files were demultiplexed and quality checked with QIIME (version 1.9.1). The readings were utilized for community diversity assessment, taxonomy classification, and OUT clustering. A standard sequence number corresponding to the sample with the fewest sequences was used to normalize the OTU abundance data. At a similarity threshold of 97%, operational taxonomic units (OTUs) were selected and identified; OTUs lacking a bacterial phylum assignment or originating from chloroplasts or mitochondria were omitted. The α -diversity revealed the richness and evenness of each sample's bacterial community. Paired T-tests were used to compare bacterial genus abundance in insecticide-treated and untreated samples.

RESULTS

Data Summary

On average, there are 49,417,842 reads in the *Cimex hemipterus* control sample. In contrast, the treated tropical bed bugs display marginally higher numbers of reads when exposed to pyriproxyfen (50,392,421), tebufenozide (50,525,885), β -cyfluthrin + imidacloprid (49,463,607) and chlorfluazuron (42,414,568). A higher species richness was revealed by the identification of a greater number of OTUs in treated bed bugs (Table 1). The data are accessibility via NCBI SRA with the identification number: PRJNA918835. Direct URL to data: <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA918835/>

Table 1: Data Summary for each bed bug sample.

| Sample/Replicate | Number of reads (bp) | Average Length(bp) |
|------------------------------------------|----------------------|--------------------|
| Control (C1) | 54,664,740 | 409.44 |
| Control (C2) | 44,170,945 | 405.54 |
| Chlorfluazuron (CH1) | 43,279,718 | 406.15 |
| Chlorfluazuron (CH2) | 41,549,419 | 408.24 |
| Pyriproxyfen (P1) | 44,158,666 | 426.17 |
| Pyriproxyfen (P2) | 56,626,176 | 424.94 |
| Tebufenozide (T1) | 46,267,932 | 407.44 |
| Tebufenozide (T2) | 54,783,838 | 409.06 |
| β -Cyfluthrin + Imidacloprid (TM1) | 50,909,020 | 406.18 |
| β -Cyfluthrin + Imidacloprid (TM2) | 48,018,195 | 405.75 |

The Shannon and Simpson indices both point to a reduced species richness in treated samples compared to untreated samples. On the other hand, for every sample, the Chao 1 indices predicted higher species richness. Overall, there is some variation in the bacterial assemblage richness across the bed bug samples. However, when comparing the sequences using various alpha diversity metrics such as Shannon, Simpson, and CHAO1, there were no significant differences (Shannon, $p = 0.254$; Simpson, $p = 0.321$; CHAO1, $p = 0.183$) between the control and treated bed bug groups. However, when comparing treated and untreated bed bugs, the rarefaction curves showed that treated bed bugs had steeper and higher curves, indicating a higher taxonomic richness (Fig. 1). There is a possibility that these samples contain additional OTUs that have not been discovered, as indicated by the curves yet to hit a plateau. This could potentially have an impact on the Shannon and Simpson indices.

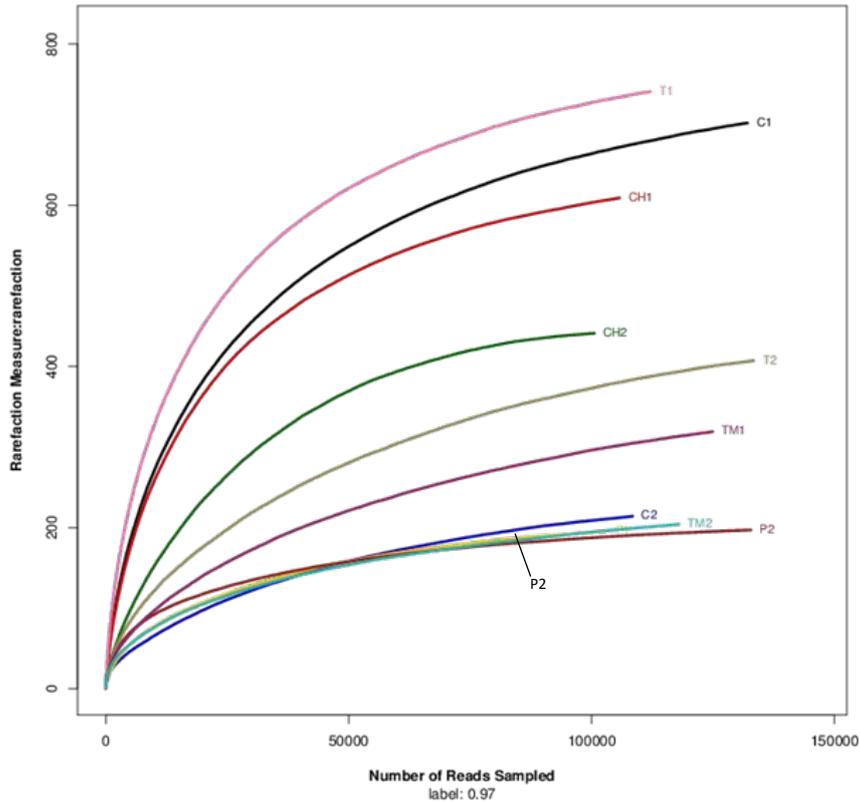


Figure 1: Rarefaction curves of control and insecticides treated tropical bed bug samples.

Microbial Community

As shown in Figure 2, the heatmap indicated that Proteobacteria constituted the predominant phylum, exhibiting relative abundances exceeding 99% in all samples. Less than 1% of the remaining OTUs were classified as belonging to a phylum Actinobacteria, Firmicutes, Fibrobacterota, Spirochaetota and others (Fig. 3). The bacterial genera/species shared between all samples were shown in Table 2.

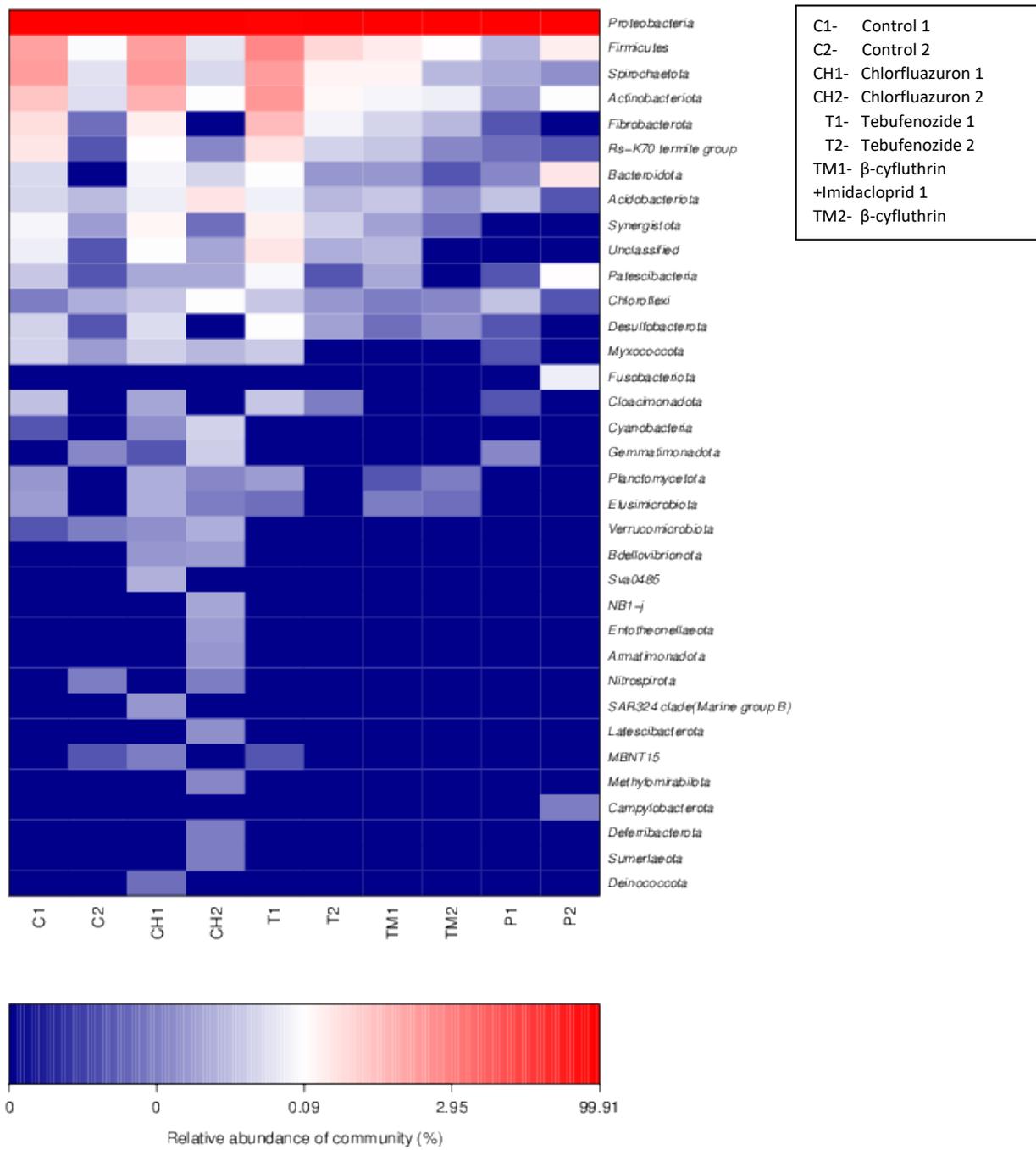


Figure 2: Heatmap analysis of ten samples, C1, C2, C1, C2, T1, T2, TM1, TM2, P1 and P2.

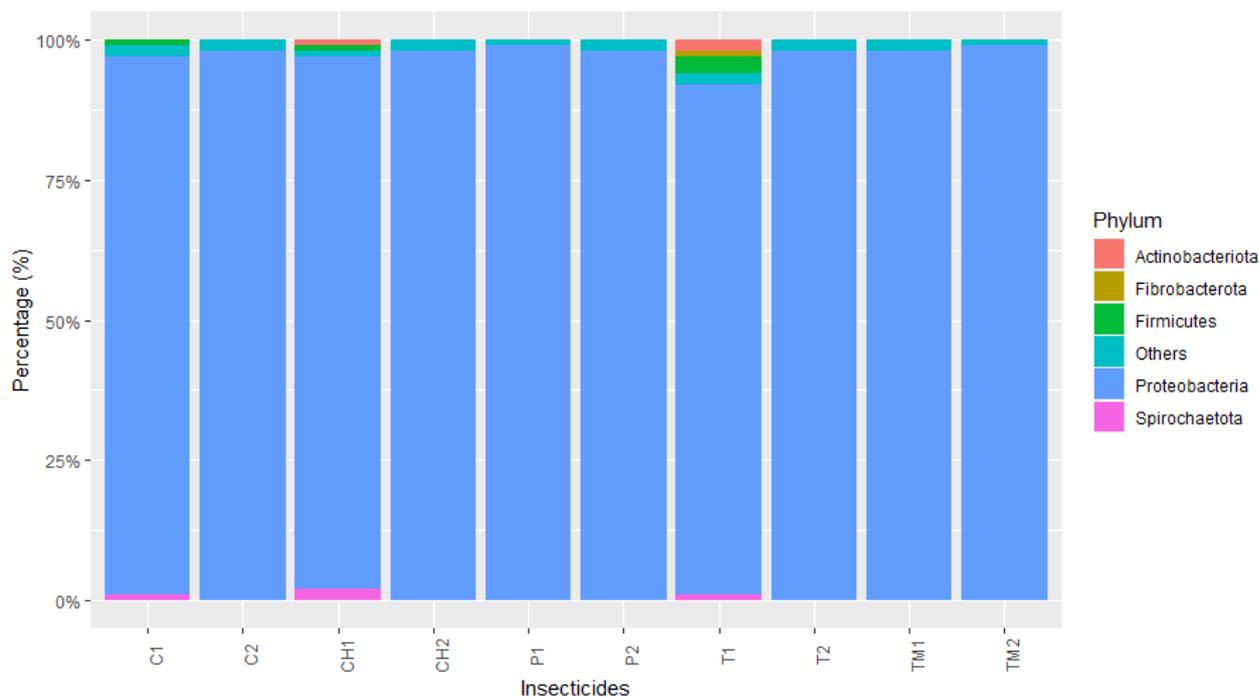


Figure 3: Summary of bacterial phyla (OTUs) of insecticides treated (CH1, CH2, P1, P2, T1, T2, TM1, TM2) and untreated (C1, C2) *Cimex hemipterus*.

The concept of the "relative abundance of identified bacterial taxa" pertains to the ratio of reads corresponding to individual taxa to the sum of all reads for all taxa. Among the bacterial genera, there were five most abundance genera, including *Wolbachia*, *Burkholderia-Caballeronia-Paraburkholderia*, *Pectobacterium*, *Sanguibacter-Flavimobilis* and Termite *Treponema* cluster were present across all samples (Fig 4). The genus, *Wolbachia* and *Burkholderia-Caballeronia-Paraburkholderia* greatly monopolized the data sequence. to be precise, *Wolbachia*, were most abundance in control sample while *Burkholderia-Caballeronia-Paraburkholderia* in pyriproxyfen treated sample (Table 2).

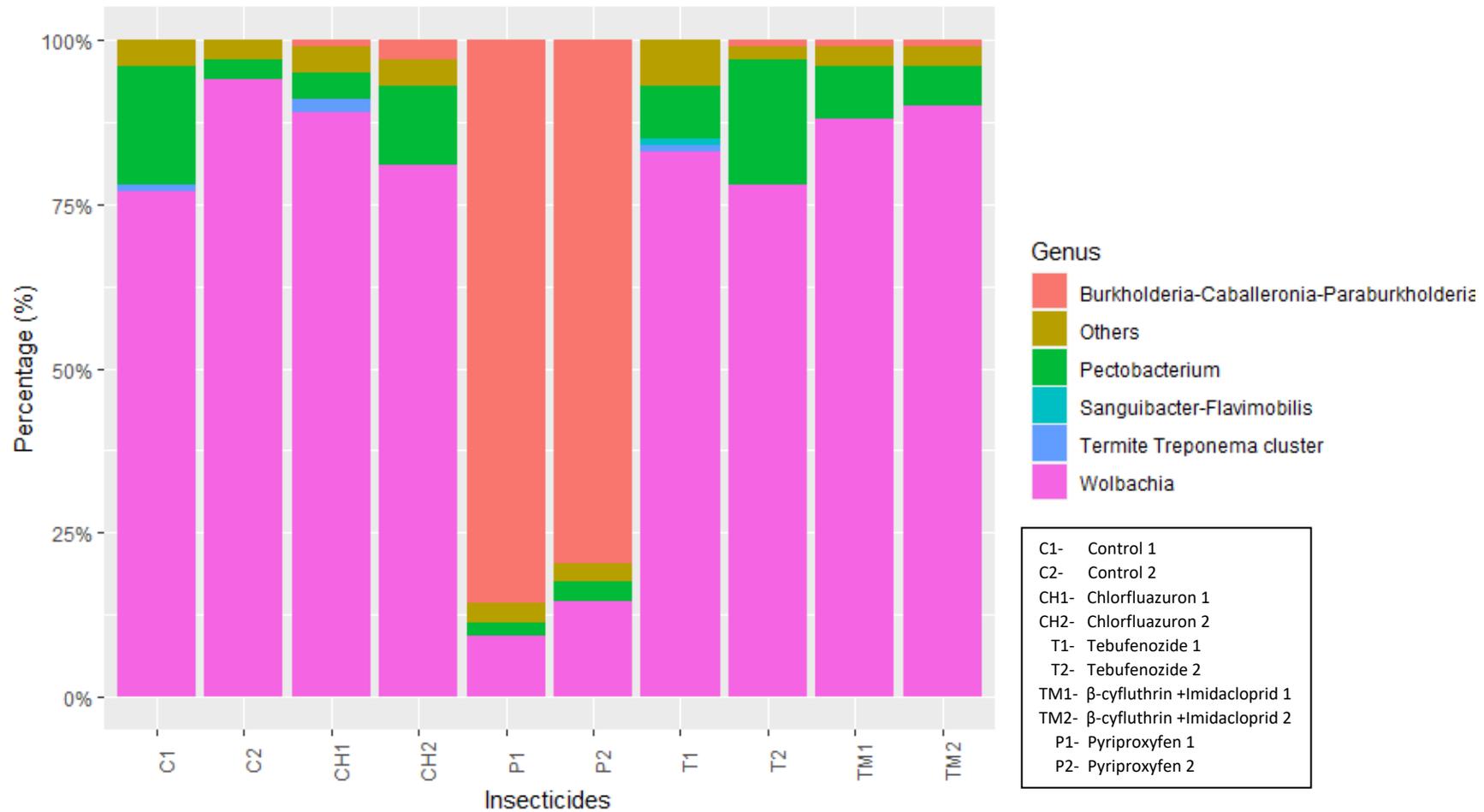


Figure 4: Summary of bacterial genera (OTUs) of insecticides treated (CH1, CH2, P1, P2, T1, T2, TM1, TM2) and untreated (C1, C2) *Cimex hemipterus*.

Table 2: Taxonomic classification of bacterial sequences (OTUs) that were shared in all 10 samples of tropical bed bugs.

| Taxonomy (Domain; phylum; class; order; family; genus; species) | Number of Sequences | | | | | | | | | |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| | C1 | C2 | CH1 | CH2 | P1 | P2 | T1 | T2 | TM1 | TM2 |
| Bacteria; Proteobacteria; Alpha proteobacteria; Rickettsiales; Anaplasmataceae; <i>Wolbachia</i> ; <i>Wolbachia-unclassified</i> | 70,276 | 49,809 | 83,166 | 73,953 | 10,008 | 14,374 | 75,322 | 70,973 | 52,509 | 80,014 |
| Bacteria; Proteobacteria; Gamma proteobacteria; Burkholderiales; Burkholderiaceae; <i>Burkholderia-Caballeronia</i> - <i>Paraburkholderia</i> ; <i>Burkholderia</i> - <i>Caballeronia-Paraburkholderia</i> - <i>unclassified</i> | 187 | 3049 | 1296 | 2797 | 86,394 | 78,892 | 235 | 654 | 724 | 1133 |
| Bacteria; Proteobacteria; Alphaproteobacteria; Rickettsiales; Anaplasmataceae; <i>Wolbachia</i> ; <i>Wolbachia pipientis</i> | 2353 | 38,817 | 1704 | 3940 | 229 | 445 | 2653 | 4121 | 33,022 | 7645 |

Paired sample T-test divulge no substantial interactions ($p > 0.005$) in bacterial sequences of insecticides treated bed bugs when compared against untreated bed bugs. The abundance ratio of the top three most abundant species of bed bugs, *Wolbachia unclassified*, followed by *Burkholderia-Caballeronia-Paraburkholderia unclassified*, and *Wolbachia pipentis*, accounted for more than 99% of the total sequences between treated and untreated bed bugs. This is despite the fact that the number of sequences of dominant genera in untreated bed bugs is relatively higher than the number of sequences pertaining to treated bed bugs. Table 2 displays a distinct differentiation in number of sequence between the two replicates used for control (C1- C2), chlorfluazuron (CH1- CH2) and B-cyfluthrin + imidacloprid (TM1-TM2) due to low quality of DNA during sample preparation.

DISCUSSION

This study focuses on characterization of the bacterial community between insecticidal treated and untreated tropical bed bugs, *Cimex hemipterus* employing sequencing via high throughput. Adaptation of an organisms to environmental condition may induce the amendment in physiological function which triggers modification in the regulation of their microbiome constituent. Symbionts play a key role in maintaining the wellness of their host as they themselves depend on the host to gain food and shelter. The study displays Proteobacteria as the predominant phylum in both treated and untreated bed bugs. The dominance of proteobacteria (> 99%) at phylum level shows no significant difference across control and untreated sample. Hence, genus level was observed (Fig. 4) to have a better illustration on the symbiont's community variations.

However, the variability between each sample showcase less than 0.1% abundance with no distinct differentiation. The alpha-diversity in all samples was relatively low due to the samples used were of the same strain and received similar blood source, although the microbial interactions or insect immune systems, plausibly affect the bacterial community's composition and proliferation rate (Boissière et al., 2012). Interestingly, this study exhibits variations between replicate to replicate which could be justify due to technicality issue during sample preparation and analysis whereby the DNA concentration may be little although the concentration and purity verified using spectrophotometer thence gel electrophoresis should be employed for a convincing result. Besides, too much primer or template DNA could also potentially influence such results (Table 2).

The findings point out that the application of insecticides has a negligible impact on the overall diversity of the bacterial community harboring tropical bed bugs. A similar result had supported this discovery where Soh & Veera Singham (2022) deduce the diversity of the bacterial community between chemical + antibiotic fed bed bugs were similar to the untreated

bed bugs. Nevertheless, despite no significant differences in alpha diversity, operational taxonomic units (OTUs) display a distinct differentiation in pyriproxyfen treated bed bugs compared to other tested samples. Theoretically, control and treated samples should display a distinct differentiation in their microbial composition. However, this study in overall reveals there were no significant dissimilarities on the microbial composition except for pyriproxyfen treated sample.

This may be due to the method of treatment employed which was surface contact that requires the bed bugs to sit on the insecticides in order them to pick up the chemical and absorb via their cuticle to reach their system. Thus, the effect of the insecticide could be minimal in influencing the microbial composition within *Cimex hemipterus*. The disclosure of bacterial genus that were present in all samples indicated that they were transmitted vertically; furthermore, their existence is vital to their hosts as they supply nutrients and support homeostasis as well of insect immunity (Hypša & Aksoy 1997; Lim & Ab Majid 2021). Among the shared bacteria, members of Proteobacteria, *Wolbachia* and *Burkholderia-Caballeronia-Paraburkholderia*. predominates the bacterial constituent of *C. hemipterus*. Although the proteobacterium found in these studies was labelled as unclassified, it is common to find alpha and gamma-proteobacteria in *C. hemipterus*, which aligns with the findings of Hypša and Aksoy 1997.

Wolbachia are an indisputable endosymbionts of *Cimex spp.* as this fauna resides within the bacteriome of *Cimex hemipterus* and act as a contractual provider of nutrient – vitamin B, biotin (B7), riboflavin (B2) aside for being liable for host reproduction (Hosokawa et al., 2010; Akhoundi et al., 2016; Bellinvia et al., 2020; Lim & Ab Majid, 2021). Fascinatingly, *Wolbachia* were uncovered to be capable in improving both male and female bed bugs' fitness by boosting egg laying and hatching rate, improving progeny body size, emergence succession and lowering wound or infection due to episodes of traumatic insemination (Heaton, 2013; Hickin et al., 2022). This study theoretically managed to classify *Wolbachia* into two strains- *Wolbachia unclassified* and *Wolbachia pipientis* where two strains mark up the plethora of gram negative alphaproteobacterial. The second most prevalent genera in this study were *Pectobacteria*, a plant hazardous enterobacteria that causes economically significant soft rot disease (Amir et al., 2020; Hugouvieux-Cotte-Pattat, 2016). This gram-negative gamma proteobacteria is likely to be represented as the secondary endosymbionts of the bed bugs (Pietri et al., 2020). Regardless, loss of *Wolbachia* and *Pectobacteria* in bed bugs has signified deceleration in development time and shrinkage in body size though it was not refined either the growth retardation was induced by these bacteria (Goodman, 2016).

Application of insecticides via contact bioassay typically affects the regulation of bacterial symbiont as microbial production either upregulated or down regulated when compared with the untreated bed bug sample. *Burkholderia-Caballeronia-Paraburkholderia*

were reported as maternally transmitted strain that are common in insect pest, they certainly presented in all sample except control (C1) and tebufenozide (T1) in this study (Peta & Pietri, 2021). But by far there are no validated findings to dictate the presences of *Burkholderia-Caballeronia-Paraburkholderia* in arthropod. Based on this study, genus *Treponema* was found in control (C1), chlorfluazuron (CH1) and tebufenozide (T1) treated samples. The *Treponema* of spirochetes class is usually found in humans, animals and insects. Exploration of biochemistry and genomic assay reveals this genus have a distinctive function in termite species where they inherently stimulate nutritional balance, energy catabolism, hydrogen scavenging in insect gut (Evans et al., 2011; Diouf et al., 2018). *Sanguibacter-flavimobilis* is a soil based facultative aerobic bacterium that was exceptionally identified from tebufenozide treated bed bug sample (Dueholm et al., 2022). The presences of this genera were customarily detected from blood of healthy cow, beef cattle and *Tenebrio Molitor* but the sequence of reads were doubtlessly low (Tye, 2023). Bed bugs in this study may have incorporated with these genera via horizontal contact of the environmental biota. From the overall taxonomic classification, could be deduced that there was no human pathogenic bed bug symbiont discovered. Basically, the symbionts identified were commonly found in the environment. Nevertheless, *Burkholderia* genus were reported found within human gut as an opportunistic pathogen, but the clade *Burkholderia-Paraburkholderia-Cabelleronia* could be classified as novel within bed bugs not to mention in humans (Mahenthiralingam et al., 2008)

Insecticides treated with different modes of action do not signify a distinct up or downregulation of endosymbionts when compared to control. However, only Juvenile hormone analogue (JHA) substantially modulate the evenness of gut mycobiome community in pyriproxyfen treated bed bugs where the titer of *Burkholderia-Caballeronia-Paraburkholderia* were relatively higher as Li et al. (2021) discloses abundance of normal flora tend to decrease but the secondary flora began to increase from the JHA treatment (Lu et al., 2022). Besides, host-driven mechanisms also could potentially induce shifts in the composition of bed bug bacterial communities where host may create altered antimicrobial proteins, pH changes, decreased mucus secretion on the intestinal lining and decreased intestine size throughout the study period (Kohl et al., 2014).

CONCLUSION

This study examined the bacterial community structure of *C. hemipterus* in two groups: one that was treated with insecticides and another that was left untreated. There was no discernible difference in the bacterial community makeup between the treated and untreated samples. Nevertheless, insecticides may have slight effect the titer of gut symbionts production in treated sample. Although the roles of some of the bacteria found in tropical bed

bugs are unknown, the results of this study help shed light on the possible roles of most bacteria in bed bugs. It is necessary to conduct additional research into the physiological characteristics, potential functions, and interactions of these bacteria with bed insect biology.

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AUTHORS DECLARATIONS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICS DECLARATIONS

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Universiti Sains Malaysia Research Ethics Committee (Human) JEPeM, Code: USM/JEPeM/19120868

DATA AVAILABILITY STATEMENT

The manuscript has no associated data.

AUTHORS' CONTRIBUTIONS

Nurhidayah Taibukahn: Methodology, Investigation, Data Curation, Formal analysis, Writing – original draft, Writing – review & editing.

Martini Martini- Conceptualization, Supervision, Writing – review & editing

Abdul Hafiz Ab Majid: Conceptualization, Supervision, Project administration, Resources, Funding acquisition, Writing – review & editing.

REFERENCES

- Akhoundi, M., Cannet, A., Loubatier, C., Berenger, J. M., Izri, A., Marty, P., & Delaunay, P. (2016). Molecular characterization of *Wolbachia* infection in bed bugs (*Cimex lectularius*) collected from several localities in France. *Parasite*, 23.
- Amir, R., Sani, Q. U. A., Maqsood, W., Munir, F., Fatima, N., Siddiqa, A., & Ahmad, J. (2020). Pan-genomics of plant pathogens and its applications. *Pan-Genomics: Applications, Challenges, and Future Prospects*, 121–145.
- Bellinvia, S., Johnston, P. R., Reinhardt, K., & Otti, O. (2020). Bacterial communities of the reproductive organs of virgin and mated common bedbugs, *Cimex lectularius*. *Ecological Entomology*, 45(1), 142–154.
- Benoit, J. B., Del Grosso, N. A., Yoder, J. A., & Denlinger, D. L. (2007). Resistance to dehydration between bouts of blood feeding in the bed bug, *Cimex lectularius*, is enhanced by water conservation, aggregation, and quiescence. *American Journal of Tropical Medicine and Hygiene*, 76(5), 987–993.
- Boissière, A., Tchioffo, M. T., Bachar, D., Abate, L., Marie, A., Nsango, S. E., & Morlais, I. (2012). Midgut microbiota of the malaria mosquito vector *Anopheles gambiae* and interactions with *Plasmodium falciparum* infection. *PLoS pathogens*, 8(5), e1002742.
- Bukin, Y. S., Galachyants, Y. P., Morozov, I. V., Bukin, S. V., Zakharenko, A. S., & Zemskaya, T. I. (2019). The effect of 16S rRNA region choice on bacterial community metabarcoding results. *Scientific Data* 2019 6:1, 6(1), 1–14.
- Doggett, S. L., Geary, M. J., & Russell, R. C. (2004). The resurgence of bed bugs in Australia: With notes on their ecology and control. *Environmental Health*, 4(2), 30–38.
- Dieng, H., Satho, T., Abang, F., Meli, N. K. K. B., Ghani, I. A., Nolasco-Hipolito, C., ... & Noweg, G. T. (2017). Sweet waste extract uptake by a mosquito vector: Survival, biting, fecundity responses, and potential epidemiological significance. *Acta tropica*, 169, 84–92.
- Diouf, M., Hervé, V., Mora, P., Robert, A., Frechault, S., Rouland-Lefèvre, C., & Miambi, E. (2018). Evidence from the gut microbiota of swarming alates of a vertical transmission of the bacterial symbionts in *Nasutitermes arborum* (Termitidae, Nasutitermitinae). *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, 111(4), 573–587.

- Dueholm, M. K. D., Nierychlo, M., Andersen, K. S., Rudkjøbing, V., Knutsson, S., Arriaga, S., Bakke, R., Boon, N., Bux, F., Christensson, M., Chua, A. S. M., Curtis, T. P., Cytryn, E., Erijman, L., Etchebehere, C., Fatta-Kassinos, D., Frigon, D., Garcia-Chaves, M. C., Gu, A. Z., Nielsen, P. H. (2022). MiDAS 4: A global catalogue of full-length 16S rRNA gene sequences and taxonomy for studies of bacterial communities in wastewater treatment plants. *Nature Communications*, 13(1).
- Evans, N. J., Brown, J. M., Murray, R. D., Getty, B., Birtles, R. J., Hart, C. A., & Carter, S. D. (2011). Characterization of Novel Bovine Gastrointestinal Tract *Treponema* Isolates and Comparison with Bovine Digital Dermatitis *Treponemes*. *Applied and Environmental Microbiology*, 77(1),
- Goddard, J., & DeShazo, R. (2009). Bed bugs (*Cimex lectularius*) and clinical consequences of their bites. *JAMA*, 301(13), 1358–1366.
- Goodman, M. H. (2016). Endosymbiotic Bacteria In The Bed Bug, *Cimex Lectularius* L. (Hemiptera: Cimicidae) [University of Kentucky]. In Theses and Dissertations--Entomology. <https://doi.org/https://doi.org/10.13023/ETD.2016.527>
- Gupta, S., Mortensen, M. S., Schjørring, S., Trivedi, U., Vestergaard, G., Stokholm, J., Bisgaard, H., Krogfelt, K. A., & Sørensen, S. J. (2019). Amplicon sequencing provides more accurate microbiome information in healthy children compared to culturing. *Communications Biology* 2019 2:1, 2(1), 1–7.
- Harlan, H. J. (2006). Bed bugs 101: the basics of *Cimex lectularius*. *American Entomologist*, 52(2), 99-101.
- Heaton, L. L. (2013). *Wolbachia* in bedbugs *Cimex lectularius* [Doctor of Philosophy, University of Sheffield]. <https://etheses.whiterose.ac.uk/5730/1/Louise%20Heaton,%20090142392,%20Wolbachia%20in%20bedbugs%20Cimex%20lectularius>.
- Hickin, M. L., Kakumanu, M. L., & Schal, C. (2022). Effects of *Wolbachia* elimination and B-vitamin supplementation on bed bug development and reproduction. *Scientific Reports*, 12(1), 1–14.
- Hosokawa, T., Koga, R., Kikuchi, Y., Meng, X. Y., & Fukatsu, T. (2010). *Wolbachia* as a bacteriocyte-associated nutritional mutualist. *Proceedings of the National Academy of Sciences of the United States of America*, 107(2), 769–774.
- Hugouvieux-Cotte-Pattat, N. (2016). Metabolism and Virulence Strategies in *Dickeya*–Host Interactions. *Progress in Molecular Biology and Translational Science*, 142, 93–129.
- Hypša, V., & Aksoy, S. (1997). Phylogenetic characterization of two transovarially transmitted endosymbionts of the bedbug *Cimex lectularius* (Heteroptera: Cimicidae). *Insect molecular biology*, 6(3), 301-304.

- Kohl, K. D., Amaya, J., Passemment, C. A., Dearing, M. D., & McCue, M. D. (2014). Unique and shared responses of the gut microbiota to prolonged fasting: a comparative study across five classes of vertebrate hosts. *FEMS microbiology ecology*, 90(3), 883-894.
- Li, F., Li, M., Zhu, Q., Mao, T., Dai, M., Ye, W., Bian, D., Su, W., Feng, P., Ren, Y., Sun, H., Wei, J., & Li, B. (2021). Imbalance of intestinal microbial homeostasis caused by acetamiprid is detrimental to resistance to pathogenic bacteria in *Bombyx mori*. *Environmental Pollution*, 289, 117866.
- Lim, L., & Ab Majid, A. H. (2021). Characterization of bacterial communities associated with blood-fed and starved tropical bed bugs, *Cimex hemipterus* (F.) (Hemiptera): a high throughput metabarcoding analysis. *Scientific Reports*, 11(1).
- Lu, Q., Li, G., Lan, H., Yu, D., Yin, X., Yang, W., Yang, Z., He, C., Mo, D., & Xu, K. (2022). Effects of exposure to trace pyriproxyfen on the intestinal bacterial diversity and immune signal pathways of silkworm (*Bombyx mori*) larvae. *Journal of Asia-Pacific Entomology*, 25(2), 101895.
- Mahenthiralingam, E., Baldwin, A., & Dowson, C. G. (2008). *Burkholderia cepacia* complex bacteria: opportunistic pathogens with important natural biology. *Journal of applied microbiology*, 104(6), 1539-1551.
- Peta, V., & Pietri, J. E. (2021). Experimental infection of bed bugs (*Cimex lectularius* L.) with *Burkholderia multivorans*. *Medical and Veterinary Entomology*, 35(3), 507–512.
- Pietri, J. E., Yax, J. A., Agany, D. D. M., Gnimpieba, E. Z., & Sheele, J. M. (2020). Body lice and bed bug co-infestation in an emergency department patient, Ohio, USA. *IDCases*, 19, e00696.
- Potter, M. F., Romero, A., Haynes, K. F., & Hardebeck, E. (2007). Killing them softly: Battling bed bugs in sensitive places. *Pest Control Technology*, 35, 24–32.
- Rupawate, P. S., Roylawar, P., Khandagale, K., Gawande, S., Ade, A. B., Jaiswal, D. K., & Borgave, S. (2023). Role of gut symbionts of insect pests: A novel target for insect-pest control. *Frontiers in Microbiology*, 14, 1146390.
- Soh, L. S., & Veera Singham, G. (2022). Bacterial symbionts influence host susceptibility to fenitrothion and imidacloprid in the obligate hematophagous bed bug, *Cimex hemipterus*. *Scientific Reports*, 12(1), 1–16.
- Tye, S. R. (2023). Interplay Between Microbiome and Temperature In *Tenebrio Molitor*. Texas State University.