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RESEARCH ARTICLE

Genetic diversity, microbiome composition and socio-sanitary predictors of head lice (*Pediculus humanus capitis*) among disadvantaged children in Klang Valley, Malaysia

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ABSTRACT

Pediculosis capitis remains a neglected public health issue in Malaysia, particularly among disadvantaged children. While the genetic diversity of head lice is well studied, their associated microbiome and links to socio-sanitary conditions remain unclear. This study examined 266 children from ten children's establishments in Klang Valley and Greater Kuala Lumpur, of whom 89 (33.46%) were positive for pediculosis capitis. Cytochrome c oxidase subunit I (*COI*) barcoding identified two clades: A (36%) and C (64%). 16S rRNA metagenomic profiling of pooled samples revealed higher microbial diversity in Clade C compared to Clade A, with opportunistic bacteria, including *Propionibacterium acnes, Streptococcus* spp., *Bacteroides fragilis*, and *Staphylococcus aureus* being detected. Logistic regression identified age, head lice awareness, and eating with hands as significant predictors of infection. These findings demonstrate that head lice not only cluster genetically but also may harbour clade-dependent microbiomes, with potential health implications. The integration of genetic diversity, microbial variation, and socio-sanitary data highlights the multifactorial risks of pediculosis capitis in vulnerable populations, underscoring the importance of combined ectoparasite control and hygiene interventions.

Keywords: *Pediculus humanus capitis*; genetic diversity; *COI*; socio-sanitary risk factors; Malaysia.

INTRODUCTION

Pediculosis capitis, caused by *Pediculus humanus capitis*, is a persistent public health issue worldwide. In Malaysia, the burden is particularly high among socioeconomically disadvantaged populations, where overcrowded living conditions and limited access to healthcare exacerbate transmission. A pooled prevalence of pediculosis capitis among primary schoolchildren in low- and middle-income countries has been estimated at nearly 20% (Delie *et al.*, 2024), with Malaysia reporting comparable rates, especially in marginalised communities (Lye *et al.*, 2017; Abd Majid *et al.*, 2020).

Genetic studies have revealed that human head lice can be divided into six mitochondrial clades (A-F) based on cytochrome c oxidase subunit I (*COI*) and cytochrome b (*cytb*) sequences, each with distinct geographical distributions. Clade A is globally distributed; clade B occurs mainly in the Americas and parts of Europe, clade C in Asia and Africa, while clades D-F have more localised distributions (Sunantaraporn *et al.*, 2015; Amanzoughaghene *et al.*, 2019). In Malaysia, only clades A, B, and C have been detected (Mokhtar *et al.*, 2020, 2021).

Beyond genetic diversity, recent research has shown that head lice harbour diverse microbial communities with potential epidemiological implications. Pathogens such as *Coxiella burnetii* and *Bartonella quintana* have been detected in head lice, along with opportunistic bacteria like *Acinetobacter baumannii*, raising concerns about head lice as reservoirs of clinically relevant microbes (Amanzougaghene *et al.*, 2017; Boumbanda-Koyo *et al.*, 2019). In Malaysia, molecular detection of *Acinetobacter* spp., *Serratia marcescens* and *Staphylococcus aureus* in head lice further supports this risk (Mokhtar *et al.*, 2020). However, the association between mitochondrial clade identity and microbial community composition remains poorly understood.

Socio-sanitary determinants, including poor hygiene, infrequent hair washing, overcrowding, reliance on unsafe water sources and close contact in overcrowded environments, are well-established drivers of head lice transmission (Moretti *et al.*, 2015). Yet, their potential role in shaping the head lice microbiome, and by extension, influencing pathogen carriage, has received little attention. This represents a critical gap, particularly in vulnerable populations where infestations and re-infestations are common.

This study aimed to address this gap by examining the genetic diversity and microbiome of head lice collected from disadvantaged children in Klang Valley and Greater Kuala Lumpur, Malaysia, while simultaneously evaluating socio-sanitary risk factors for infestation. By integrating molecular and epidemiological data, we sought to provide novel insights into the host-parasite-microbe interactions and highlight their implications for public health interventions.

MATERIALS AND METHODS

Ethical Approval and Study Sites

This study was approved by the UMMC Medical Research Ethics Committee (MEC ID: 2024813-14045). Informed assent was obtained from participating children, with written consent provided by their legal guardians. Head lice sampling was conducted between October 2024 and January 2025 across ten establishments in Klang Valley and Greater Kuala Lumpur, Malaysia. These comprised six establishments that house orphans and neglected children, while the remaining four serve as learning centres for refugees from diverse ethnic backgrounds. All establishments were selected based on accessibility, willingness to participate, representation of underprivileged populations, and to cover a dispersed location within the targeted region (Klang Valley/Greater Kuala Lumpur).

The required sample size was calculated using OpenEpi v3.01 based on the "Frequency in a Population (Random Sample)" module. Using a 95% confidence level, 5% confidence limit, and an anticipated prevalence (p) of 14% based on a recent local study of head lice infestation (Mokhtar *et al*., 2021), and assuming a design effect of 1.0, the minimum required sample size was 185 participants. To account for potential missing data and an estimated 30% non-response rate, the sample size was adjusted using the formula:

$$\text{Adjusted sample size} = 185 / (1 - \text{non-response rate})$$
$$= 185 / 0.7$$
$$= 265$$

The final achieved sample size in this study was 266 children, which met the calculated target.

Socio-Sanitary Risk Factor Assessment and Statistical Analysis

A structured questionnaire was administered to all participating children to obtain information on socio-demographic characteristics, environmental exposures, hygiene practices, awareness of head lice, and hair-related factors. As the questionnaire was designed for this study and not formally validated, responses may be subject to reporting bias. However, items were developed based on published risk factors (Moretti *et al.*, 2015; Lye *et al.*, 2017) and were administered by trained interviewers to minimise misinterpretation.

Questionnaire data were analysed using IBM SPSS Statistics (Version 30). The infestation status was treated as a binary dependent variable, with infested (1) and non-infested (0) categories. Univariable analyses were performed using simple logistic regression to examine associations between infestation and individual explanatory variables. Multiple logistic regression was then performed using stepwise backward likelihood ratio elimination to identify independent predictors of infestation while controlling for potential confounders. Model fit was confirmed by the Hosmer-Lemeshow test. Crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for factors associated with head lice infestation, with significance set at $p < 0.05$.

Head Lice Sampling and Processing

A total of 266 children aged 4-18 years were examined for head lice infestation. Examination was conducted under natural light using fine-tooth combs, with infestation defined by the presence of viable nits, nymphs, or adult head lice. Each child's hair was systematically combed from scalp to tip, and all lice retrieved were immediately transferred into 1.5 mL microcentrifuge tubes containing 70% ethanol for preservation.

To remove surface contaminants, representative louse from each child per establishment were sequentially rinsed in 1% sodium hypochlorite (bleach), followed by 70% ethanol, and then in sterile distilled water and stored at –20°C until DNA extraction after the samples were dried; all according to established protocols (Binetruy *et al.*, 2019). All samples were treated individually, which were taken from the 70% ethanol vial. The head lice were first rinsed in distilled water and dried on a sterile filter paper. An additional step of crushing the samples with blunt pipette tips to ensure homogenisation before DNA extraction. Genomic DNA was extracted from individual head louse per child using the PrimeWay Genomic II Kit (Apical Scientific Sdn. Bhd., Malaysia), following the manufacturer's protocol for insects. DNA quality and concentration were assessed using NanoQuant Infinite® M200 (Tecan, Switzerland). Extracted DNA was stored at –20°C.

PCR Amplification and Sequencing of the COI Gene

The mitochondrial cytochrome c oxidase subunit I (*COI*) partial sequences in head lice were amplified using the forward primer 5'-GGTACTGGCTGGACTRTTTATCC-3' and reverse primer 5'-CTAAARACTTTYACTCCCGTTGG-3' as previously described by Sunantaraporn *et al.* (2015). PCR reactions were 20 µL containing 10 µL PCR Master Mix (2X) (Thermo Fisher Scientific, United States), 1 µL of both forward primer and reverse primer each, 7 µL sterile distilled water, and 1 µL template DNA. Cycle sequencing was performed SuperCycler thermal cycler (Kyratec, Australia), using the PCR primers by Apical Scientific Sdn. Bhd. (Selangor, Malaysia).

***COI* Sequence Analysis and Phylogenetic Tree Construction**

The sequences obtained were aligned using the ClustalW algorithm in BioEdit Sequence Alignment Editor Software version 7.2.6.1 (Hall, 1999). Pairwise genetic distances for the *COI* gene were computed with the Kimura two-parameter method (K2P). Maximum Likelihood phylogenetic trees were constructed using 1000 bootstrap replications to assess branch support in MEGA 12 (Kumar *et al.* 2024).

16S rRNA Metagenomic Sequencing and Bioinformatic Analysis

For microbial profiling, the genomic DNA extracts of head lice were pooled according to the mitochondrial clade and establishment, resulting in 11 DNA pools. Pooling was employed to maximise DNA yield and reduce sequencing costs while preserving clade- and site-specific representation. The V3–V4 hypervariable region of the bacterial 16S rRNA gene was amplified using primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3'). Amplicon libraries were prepared and sequenced on the Illumina MiSeq platform (2 × 300 bp paired end) by AGTC Genomics Sdn. Bhd. (Kuala Lumpur, Malaysia).

Raw sequencing reads were processed using CLC Genomics Workbench v24 (QIAGEN, Germany). Quality trimming was performed using default parameters, with a quality limit of 0.05 and ambiguous nucleotides exceeding two bases were trimmed. Automatic adapter removal was enabled, and trimming was applied from the 3' end of each read. Both forward and reverse reads were processed.

OTU clustering was performed using reference-based OTU clustering against the Greengenes database (v13.8). Default parameters were applied, allowing the creation of new OTUs for sequences not represented in the reference set, with a taxonomy similarity threshold of 97% and a minimum occurrence of 2. Alignment scoring parameters included a k-mer size of 6, mismatch cost = 1, gap cost = 4, chimaera crossover cost = 3, maximum unaligned end mismatches = 5, and minimum score = 40. Chimaera detection was performed using the UCHIME algorithm, and an abundance table and OTU sequence list were generated. Alpha-diversity indices (Shannon) and beta-diversity (Bray–Curtis dissimilarity, Principal Coordinates Analysis [PCoA]) were computed to assess within- and between-sample diversity across clades.

RESULTS

Prevalence of Pediculosis Capitis

Head lice were collected via convenient sampling from ten children's establishments in Klang Valley and the Greater Kuala Lumpur region. Sampling locations were widely distributed, as shown in Figure 1. Prevalence varied by establishment, ranging from 0% at Persatuan Kebajikan Wen Hua to 100% at Rumah K.I.D.S (Figure 2). Overall, 89 of 266 children (33.46%) were infested.

Socio-Sanitary Risk Factor Analysis

Children aged 10–18 years were more likely to be infested (crude OR = 1.21, 95%CI = 3.32–5.98, p<0.001) (Table 1). Female gender was strongly associated with infestation (cOR = 7.00, 95% CI: 3.31–14.83, p < 0.001). Environmental exposures, including use of river water (cOR = 12.27, p < 0.001) and contact with domestic or stray animals (cOR = 2.62, p = 0.003), also significantly increased odds of infestation. The mean hygiene composite score across all children was 6.06 (SD = 1.26) (Table 2). Infested children had slightly lower scores, but the difference was not statistically significant (cOR = 0.95, 95% CI: 0.78–1.16, p = 0.603).

Children who reported the desirable practice of not eating with their hands have a 69% lower likelihood of infestation (cOR = 0.31, 95% CI: 0.16–0.57, p < 0.001). Hair characteristics were also associated with infestation. Long hair (cOR = 2.00, 95% CI: 1.05–3.81, p = 0.034) and thick hair (cOR = 1.83, 95% CI: 1.09–3.07, p = 0.022) increased the likelihood of infestation (Table 3).

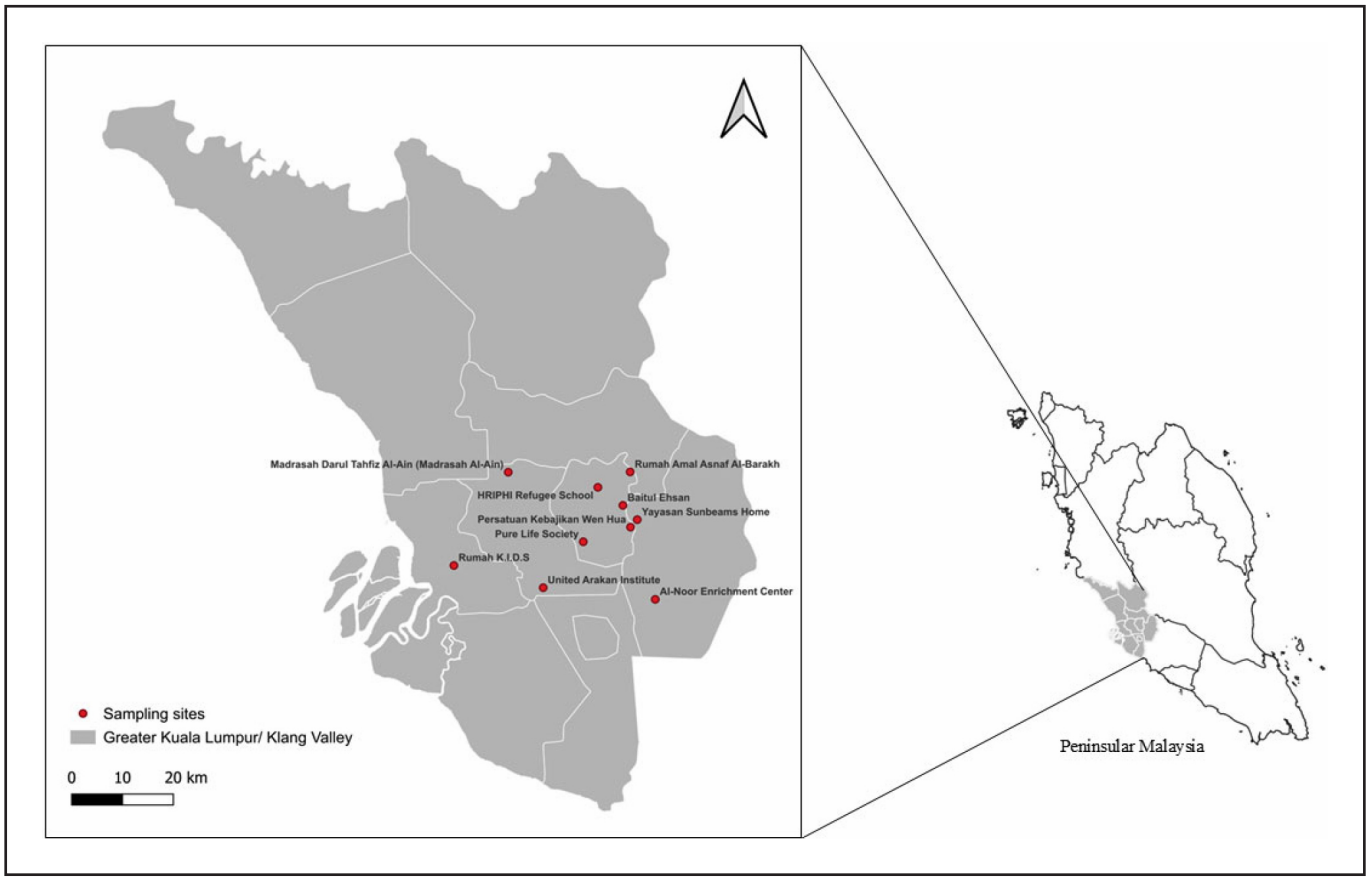

Figure 1. Map of head lice sampling in children's establishments in Greater Kuala Lumpur/ Klang Valley (KL/KV) of Peninsular Malaysia. Ten children's establishments from five cities in Greater KL/KV are included in this study. The red circular symbol shows the coordinates of each establishment generated via QGIS v3.40.7.

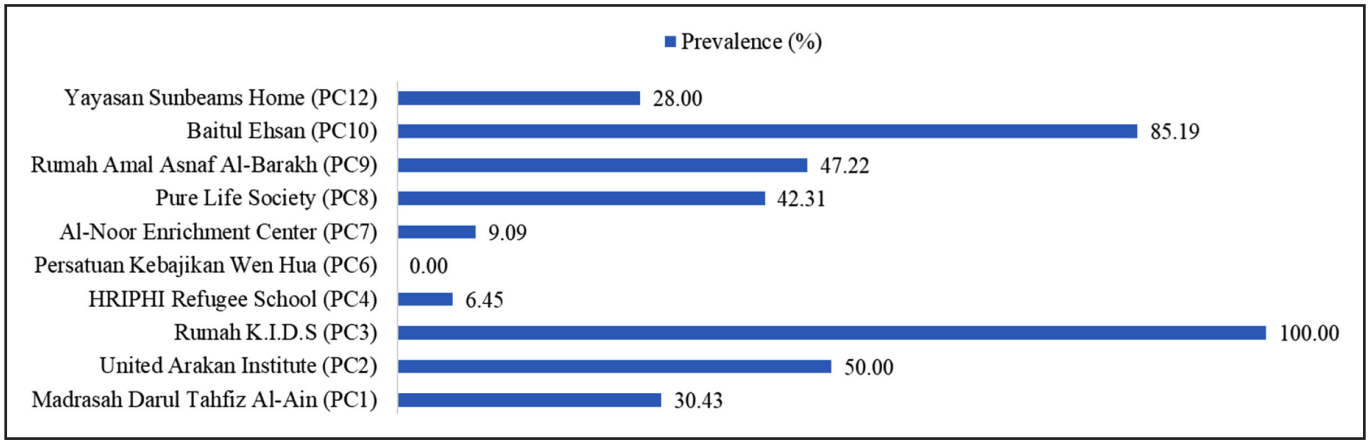

Figure 2. The bar plot shows the prevalence of pediculosis in shelter establishments across Greater KL/KV.

Table 1. Demographic characteristics, environmental, and sanitation factors and their association with head lice infestation, by simple logistic regression (N=266)

Characteristics/Factors	n (%)	Crude OR (95% CI)	*p*-value
Demographic characteristics			
Age (years), n = 264 [a]	10.81 (3.30)	1.18 (1.08, 1.28)	**<0.001**
10–18	162 (61.26)	1.21 (3.32, 5.98)	**<0.001**
4–9	102 (38.64)	1	
Gender			
Female	179 (67.29)	7.00 (3.31, 14.83)	**<0.001**
Male	87 (32.71)	1	
Number of siblings, n = 221 [b]	3.00 (2.00)	1.11 (0.95, 1.30)	0.179
Environmental and sanitation factors			
Source for water supply			
Government pipe water	252 (94.74)	1	
River	13 (4.89)	12.27 (2.66, 56.67)	**<0.001**
Well	1 (0.38)	NE[c]	NE[c]
Presence of latrine in the house			
Yes	265 (99.62)	NE[c]	NE[c]
No	1 (0.38)		
Type of toilet facility			
Pour toilet	63 (23.68)	1	
Flush toilet	202 (75.94)	0.50 (0.28, 0.89)	**0.018**
Others	1 (0.38)	NE[c]	
Defecation site			
Toilet	265 (99.62)	NE[c]	NE[c]
Pit or hole	1 (0.38)		
Garbage disposal			
Collected	261 (98.12)	1	
Indiscriminately	5 (1.88)	0.49 (0.05, 4.46)	0.528
Close contact with a domestic or stray animal			
Yes	48 (18.05)	2.62 (1.38, 4.95)	**0.003**
No	218 (81.95)	1	

Footnote: [a] Mean (SD); [b] Median (IQR); [c] Not estimable due to sparse cell counts.

Table 2. Behavioural hygiene practices and their associations with the head lice infestation, by simple logistic regression (N=266)

Factors	n (%)	Crude OR (95% CI)	*p*-value
Do you eat with your hands? †	90 (33.83)	0.31 (0.16, 0.57)	**<0.001**
Do you cut your nails at least once a week?	241 (90.60)	2.86 (0.95, 8.61)	0.061
Do you bathe at least once a day?	245 (92.11)	2.26 (0.74, 6.92)	0.154
Do you change your clothes at least once a day?	242 (90.98)	1.01 (0.41, 2.45)	0.989
Do you wear shoes when you go outside?	252 (94.74)	3.16 (0.69, 14.46)	0.137
Do you wash your hands before eating?	240 (90.23)	1.76 (0.68, 4.56)	0.243
Do you wash your hands after going to the toilet?	245 (92.11)	0.80 (0.32, 2.01)	0.639
Do you share your comb? †	126 (47.37)	0.81 (0.48, 1.35)	0.411
Do you share your towel? †	170 (63.91)	1.26 (0.74, 2.16)	0.399

Footnote: Percentages represent correct responses “Yes” for all items unless marked †, where “No” indicates a desirable hygiene practice.

Narrative:
Mean total hygiene is 6.06 (1.26).
Association with head lice infestation by simple logistic regression, OR: 0.95 (95% CI: 0.78, 1.16; p-value:0.603).

Table 3. Knowledge and hair-related factors to head lice infestation, by simple logistic regression (N=266)

Factors	n (%)	Crude OR (95% CI)	*p*-value
Do you know what head lice is?			
Yes	150 (56.39)	2.15 (1.26, 3.68)	**0.005**
No/Unsure	116 (43.61)	1	
Frequency of hair washing			
Once a week or less	38 (14.29)	0.91 (0.43, 1.89)	0.791
Twice a week	228 (85.71)	1	
Hair length			
Short	143 (53.76)	1	
Medium	68 (25.56)	1.15 (0.62, 2.14)	0.660
Long	55 (20.68)	2.00 (1.05, 3.81)	**0.034**
Hair style			
Straight	168 (63.16)	1	1
Curly	42 (15.79)	1.28 (0.63, 2.60)	0.504
Wavy	56 (21.05)	1.60 (0.86, 2.99)	0.142
Hair thickness			
Thick	135 (50.75)	1.83 (1.09, 3.07)	**0.022**
Thin	131 (49.35)	1	
Dandruff problem			
No	133 (50.00)	1.36 (0.81, 2.26)	0.243
Yes	133 (50.00)	1	

Table 4. Demographic characteristics, environmental, sanitation, hair-related and behavioural hygiene practice factors associated with head lice infestation by multiple logistic regression (N=266)

Factors	n (%)	Adjusted OR (95% CI) [b]	*p*-value
Age (years), n = 220 [a]	10.75 (3.13)	1.14 (1.04, 1.24)	**0.004**
Head lice awareness	150 (56.39)	1.98 (1.13, 3.47)	**0.017**
Eating with hands	176 (66.17)	2.74 (1.44, 5.20)	**0.002**

Footnote: [A] Mean (SD); [b] Model fitness analysis: Model $X^2(3)$ =31.99, p < 0.001; Nagelkerke R^2 = 0.16; Hosmer–Lemeshow $X^2(8)$ = 14.04, *p*=0.081; Overall classification accuracy = 68.2%; AUC = 70.8% (95% CI 64.3–77.2). Multicollinearity was not a concern (all predictors had tolerance > 0.9 and VIF < 2).

In the multivariable model (Table 4), older-aged children (aOR = 1.14, 95% CI: 1.04–1.24, p = 0.004), awareness of head lice (aOR = 1.98, 95% CI: 1.13–3.47, p = 0.017), and eating with hands (aOR = 2.74, 95% CI: 1.44–5.20, p = 0.002) remained significantly associated with infestation. These findings highlight behavioural and demographic factors that independently contribute to infestation risk.

COI Head Lice Clade Distribution Analysis

Phylogenetic analysis of *COI* sequences categorised the representative head lice into two mitochondrial clades: A and C, using reference sequences from GenBank (Figure 3). Clade A was more common in PC1, PC2, PC7, PC9, and PC12, whereas Clade C predominated in PC3, PC4, PC8, PC9, and PC10 (Table 5). The co-occurrence of both clades in PC1 and PC9 indicates possible circulation of genetically distinct lineages within the same establishments, likely reflecting diverse ethnic backgrounds of the children.

16S rRNA Metagenomic Microbial Diversity Analysis According to Head Lice Clades

The 89 head lice samples were pooled by establishment and clade, resulting in 11 DNA pools (Table 5). Pooling was performed to maximise DNA yield and reduce sequencing costs, though this approach may limit resolution at the individual level. 16S rRNA gene sequencing showed that both clades shared the same five dominant bacterial classes: *Gammaproteobacteria*, *Clostridia*, *Bacteroidia*, *Erysipelotrichi*, and *Actinobacteria* (Figure 4). Within this shared profile, Clade A head lice carried higher proportions of *Gammaproteobacteria* and *Erysipelotrichi*, whereas Clade C head lice were enriched in *Clostridia* and *Bacteroidia*. Microbial diversity was further assessed using Shannon entropy (alpha-diversity) and Bray-Curtis's dissimilarity (beta-diversity). Shannon entropy indicated that Clade C head lice harboured higher microbial diversity than Clade A (Figure 5). Bray-Curtis PCoA demonstrated distinct clustering by clade (Figure 6), supporting clade-dependent differences in microbiome composition. Clade C samples clustered more tightly, whereas Clade A samples were more dispersed.

16s rRNA Metagenomic Detection of Medically Important Bacteria in Head Lice

Table 6 summarises the detection of medically important bacterial species or genera across clades and establishments. Detected bacterial species across multiple establishments are *B. eggerthii* (0.13–2.02%), *B. ovatus* (0.03-0.53%), *B. uniformis* (0.19–7.10%), *P. stercorea* (0.06–5.24%), *P. acnes* (0.01–0.31%), *Ralstonia* spp. (0.01–0.26%), *R. gnavus* (0.04–0.58%), *Streptococcus* spp. (0.48–2.57%) and *V. dispar* (0.08–2.01%). These bacterial species & genera were detected in multiple establishments, indicating consistent

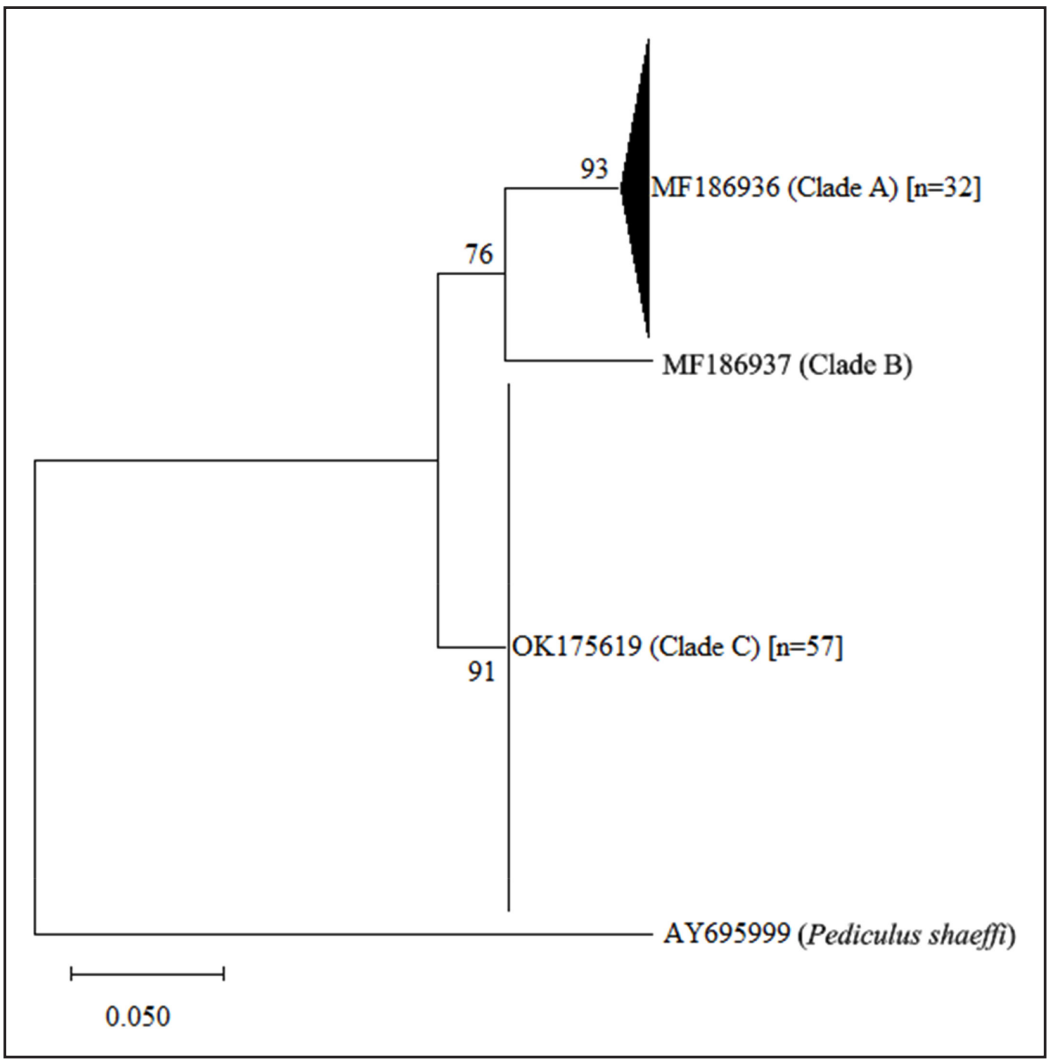

Figure 3. Maximum Likelihood (ML) cluster analysis based on partial *COI* sequences showing the phylogenetic placement of the 89 head lice into accession IDs of Clade A, B and C. Bootstrap analysis was performed with 1000 replications. The scale bar shows K2P distances. *Pediculus schaeffi* (Accession Number: AY695999) was used as an outgroup.

Table 5. Establishments, locations, and ethnic backgrounds of children sampled for head lice. Pool IDs and the number of representative head lice samples pooled from each establishment were designated and subsequently included in molecular and phylogenetic analyses to determine the distribution of clades

Establishment Code	City	Establishment	Ethnicity	Pool ID	No. of Head Lice Pooled	Total Reads Count
PC1	Sungai Buloh	Madrasah Darul Tahfiz Al-Ain (Madrasah Al-Ain)	Cambodian Malay (Champa & Khmer)	PC1_A PC1_C	1 6	903 690 487 506
PC2	Puchong	United Arakan Institute	Rohingya	PC2_A	2	566 398
PC3	Klang	Rumah K.I.D.S	Indian	PC3_C	8	272,926
PC4	Kuala Lumpur	HRIPHI Refugee School	Burmese (Hriphi, Myanmar)	PC4_C	2	345 142
PC7	Kajang	Al-Noor Enrichment Center	Arab	PC7_A	5	262 376
PC8	Kuala Lumpur	Pure Life Society	Indian & Chinese	PC8_C	11	328 716
PC9	Kuala Lumpur	Rumah Amal Asnaf Al-Barakh	Malay/Indonesian	PC9_A PC9_C	10 7	451 332 406 162
PC10	Kuala Lumpur	Baitul Ehsan	Malay	PC10_C	23	341 428
PC12	Kuala Lumpur	Yayasan Sunbeams Home	Chinese, Indian & Orang Asli	PC12_A	14	313 950
		Total			**89**	**4 679 626**

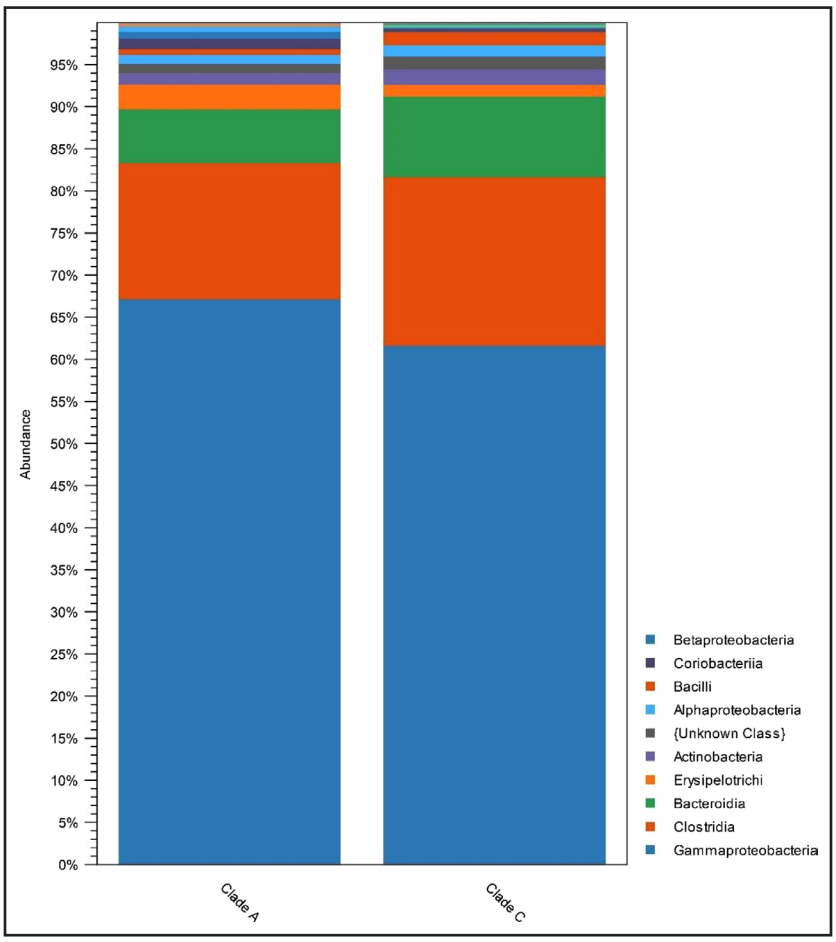

Figure 4. Relative abundance of bacterial populations at the class level in pooled head lice samples according to their respective clades: Clade A and Clade C.

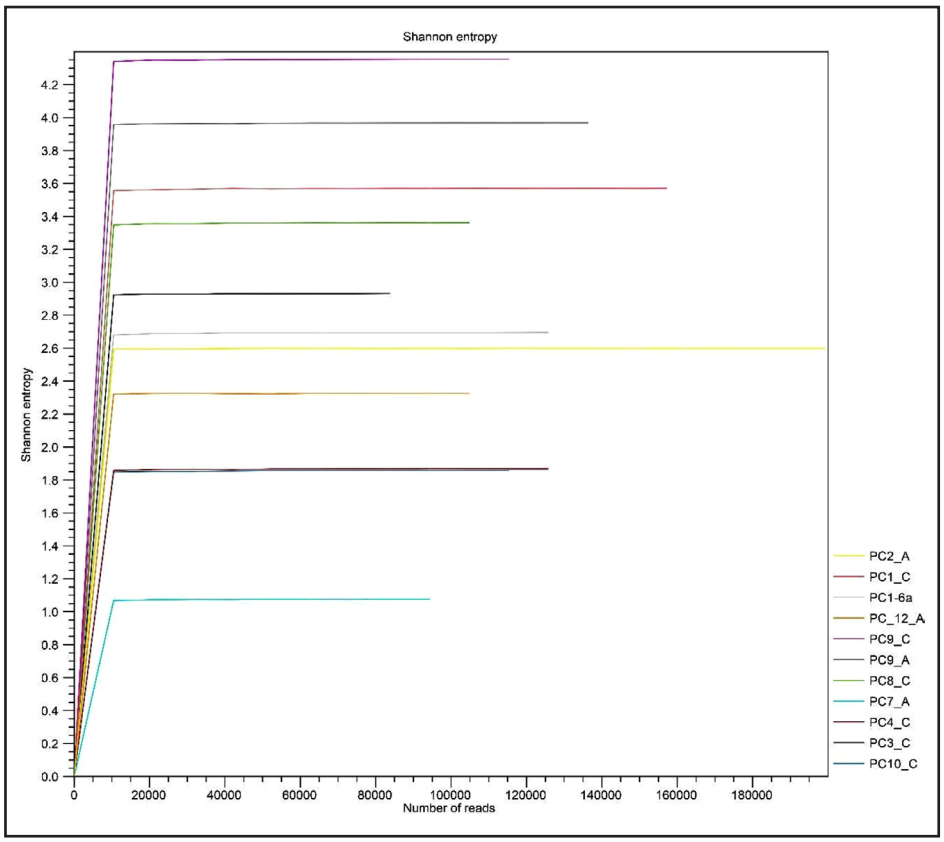

Figure 5. Line graph of Shannon Entropy for each of the pooled head lice samples against their respective number of reads. It measures both species' richness (number of different species or taxa) and evenness (the relative abundance of each species).

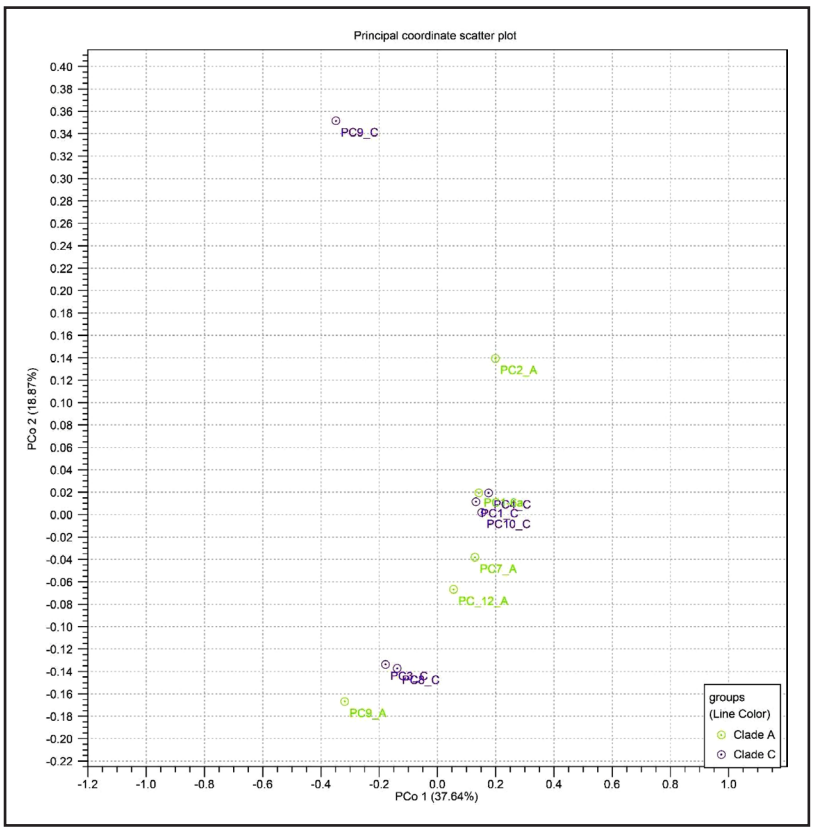

Figure 6. Principal Coordinates Analysis (PCoA) plot based on Bray–Curtis's dissimilarity of bacterial communities from pooled head lice samples. Each point represents a pooled sample, with colours indicating lice clades (green = Clade A, purple = Clade C). The x-axis (PCo 1) explains 37.64% of the variation, while the y-axis (PCo 2) explains 18.87%. Bray–Curtis's dissimilarity measures differences in microbial community composition by incorporating the relative abundance of taxa.

Table 6. Relative abundance of medically important bacterial species and genus in head lice pools of each establishment and clades. The abundance values are presented in percentage (%)

Bacterial Taxonomy	Relative Abundance (%)										
	PC1_A	PC1_C	PC2_A	PC3_C	PC4_C	PC7_A	PC8_C	PC9_A	PC9_C	PC10_C	PC12_A
Arcobacter cryaerophilus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.44**	0.00
Bacillus cereus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**0.10**	0.00
Bacteroides coprophilus	0.00	0.00	0.00	0.00	0.00	**0.03**	0.00	0.00	0.00	0.00	**0.02**
Bacteroides eggerthii	**0.13**	**0.93**	**0.32**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**2.02**
Bacteroides fragilis	0.00	0.00	0.00	**2.01**	0.00	0.00	0.00	0.00	**6.07**	0.00	0.00
Bacteroides ovatus	**0.03**	0.00	0.00	0.00	**0.53**	0.00	0.00	0.00	0.00	0.00	0.00
Bacteroides plebeius	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**1.00**
Bacteroides uniformis	**0.19**	**0.64**	0.00	**2.00**	0.00	0.00	0.00	0.00	**7.10**	0.00	0.00
Eggerthella lenta	**0.09**	0.00	0.00	0.00	0.00	0.00	0.00	**2.00**	0.00	0.00	0.00
Haemophilus parainfluenzae	0.00	0.00	0.00	0.00	0.00	**0.01**	0.00	0.00	0.00	0.00	0.00
Methylobacterium adhaesivum	0.00	0.00	0.00	**4.00**	**0.01**	0.00	0.00	0.00	0.00	0.00	0.00
Parabacteroides distasonis	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**2.12**
Prevotella nanceiensis	0.00	**0.35**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Propionibacterium acnes	**0.03**	**0.05**	0.00	0.00	0.00	**0.01**	0.00	0.00	**0.23**	**0.02**	**0.31**
Ralstonia spp.	0.00	0.00	0.00	0.00	0.00	**0.01**	**0.26**	0.00	0.00	0.00	0.00
Ruminococcus gnavus	**0.32**	<0.01	**0.58**	0.00	0.00	0.00	**0.50**	0.00	0.00	**0.04**	0.00
Staphylococcus aureus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	**3.00**	0.00	0.00
Staphylococcus spp.	0.00	0.00	0.00	0.00	**0.21**	0.00	0.00	0.00	**0.01**	0.00	0.00
Streptococcus spp.	**0.53**	**0.54**	**0.48**	0.00	0.00	**0.62**	0.00	0.00	**2.57**	**1.91**	0.00
Veillonella dispar	**0.08**	0.00	0.00	**0.66**	0.00	**0.71**	0.00	0.00	**2.01**	**1.08**	0.00
Veillonella parvula	0.00	0.00	0.00	<0.01	0.00	0.00	0.00	0.00	0.00	**0.08**	0.00
Vibrio harveyi	**0.45**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Footnote: Values of >0.01% are highlighted in bold.

carriage across clades. Single-establishment detections included *A. cryaerophilus* in PC10_C (0.44%), *B. cereus* in PC10_C (0.10%), *B. plebeius* in PC12_A (1.00%), *H. parainfluenzae* in PC7_A (0.01%), *P. distasonis* in PC12_A (2.12%), *P. nanceiensis* in PC1_C (0.35%), *S. aureus* in PC9_C (3.00%), and *V. harveyi* in PC1_A (0.45%). Other species that were only detected in clade A pooled samples are *B. coprophilus* in PC7_A (0.03%) and PC12_A (0.02%), and *E. lenta* in PC1_A (0.09%) and PC9_A (2.00%). While in clade C's pooled samples are *B. fragilis* in PC3_C (2.01%) and PC9_C (6.07%), *M. adhaesivum* in PC3_C (4.00%) and PC4_C (0.01%), *Staphylococcus* spp. in PC4_C (0.21%) and PC9_C (0.01%), *V. parvula* in PC3_C (<0.01%) and PC10_C (0.08%). Given their very low relative abundance in some cases (<1%), these findings should be interpreted cautiously and may partly reflect background contamination.

DISCUSSION

The integration of mitochondrial clade identification, microbial profiling, and socio-sanitary data provides valuable insights into the ecology of head lice among disadvantaged populations in Klang Valley and the Greater Kuala Lumpur. The geographic distribution of collection sites further reflects the heterogeneity of settings in which infestations persist.

In the crude analysis, children aged 10–18 years were at higher risk of infestation, consistent with previous reports of increased prevalence among school-aged groups (Delie *et al.*, 2024). Female gender also emerged as a strong risk factor, likely reflecting longer hair and closer social contact (Delie *et al.*, 2024). Environmental exposures such as the use of river water and contact with animals were also significant, suggesting additional pathways for infestation that merit further investigation. Although mean hygiene composite scores were lower among infested children, the difference was not statistically significant. In contrast, specific behaviours such as not eating with hands were protective, underscoring the role of personal hygiene. Hair length and thickness also increased risk, supporting biological plausibility by increasing surface area for head lice transmission (Valero *et al.*, 2023). These findings highlight both biological and behavioural contributions to infestation risk.

Multivariable analysis confirmed that age, head lice awareness, and eating with hands were independent predictors of infestation. Each one-year increase in age was associated with a 14% rise in odds of infestation (aOR = 1.14, 95% CI: 1.04–1.24, p = 0.004), consistent with increased exposure through communal interactions (Chosidow, 2000; Mahmud *et al.*, 2011). Reported awareness of head lice nearly doubled the odds of infestation (aOR = 1.98, 95% CI: 1.13–3.47, p = 0.017). While counterintuitive, this may reflect that awareness arises in high-infestation communities where reinfestation is common, but preventive practices are lacking (Baghdadi *et al.*, 2021). This highlights the need to distinguish between awareness and actionable knowledge. Eating with hands was the strongest predictor, tripling infestation odds (aOR = 2.74, 95% CI: 1.44–5.20, p = 0.002). This behavioural factor may facilitate both ectoparasite transmission and exposure to contaminated fomites, particularly in communal living settings. The consistency of findings across both crude and adjusted models strengthens their validity.

The genetic analysis revealed mixed clade distributions, particularly at PC1 and PC9, where both Clade A and Clade C head lice were detected. This finding aligns with previous Malaysian studies showing co-occurrence of multiple clades, likely reflecting migration and cultural intermixing (Mokhtar *et al.*, 2021). The predominance of Clade C (~67%) is consistent with its broader distribution across Southeast Asia (Sunantaraporn *et al.*, 2015; Mokhtar *et al.*, 2021).

Microbiome profiling showed that head lice harboured diverse bacterial taxa, with community composition varying by clade. The dominant bacterial classes across both clades were *Gammaproteobacteria*, *Clostridia*, *Bacteroidia*, *Erysipelotrichi*, and *Actinobacteria* (Figure 4). Clade A head lice contained higher proportions of *Gammaproteobacteria* and *Erysipelotrichi*, while Clade C head lice were enriched in *Clostridia* and *Bacteroidia*. This clade-dependent variation is consistent with reports from Southeast Asia and Africa, suggesting that head lice phylogenetic lineage influences microbial composition (Sunantaraporn *et al.*, 2015; Amanzougaghene *et al.*, 2017). In Malaysia, previous studies have also identified potential pathogens such as *Acinetobacter* spp. and *Staphylococcus* spp. in head lice (Abd Majid *et al.*, 2020; Mokhtar *et al.*, 2020), supporting the epidemiological relevance of these associations. Shannon entropy analysis demonstrated greater microbial diversity in Clade C compared to Clade A (Figure 5). Bray-Curtis PCoA also showed distinct clustering of Clade C samples, whereas Clade A samples were more dispersed (Figure 6). These results suggest clade-dependent differences in microbial community structure. Clade C in this study also harboured several opportunistic bacteria (Table 6), highlighting its potential role in pathogen carriage.

Table 6 shows the detection of medically important bacteria across establishments and clades. *B. eggerthii, B. ovatus, B. uniformis, P. stercorea, P. acnes, Ralstonia* spp., *R. gnavus, Streptococcus* spp. and *V. dispar* were detected in multiple establishments, suggesting a consistent association with head lice microbiota. *P. acnes*, *Ralstonia* spp. and *Streptococcus* spp. have previously been reported in Malaysian Orang Asli head lice (Abd Majid *et al.*, 2020). *Bacteroides* spp. (Wexler, 2007), *P. stercorea* (Yeoh *et al.*, 2022) and *R. gnavus* (Crost *et al.*, 2023) are comprised in a normal human gut microbiome; however, they have opportunistic capabilities to affect gut health. However, for *Bacteroides* spp., there have been reports of isolates in abscesses in the abdomen, brain and lungs (Wexler, 2007). *V. dispar* is also an intestinal commensal but has been reported to cause bacteremia (Gupta *et al.*, 2025). *Ralstonia* spp., which are commonly found in the environment, have also been reported to cause an opportunistic infection in hospital settings (Ryan & Adley, 2013). *P. acnes* (McDowell *et al.*, 2013) and *Streptococcus* spp. (Krzyściak *et al.*, 2013; Di Pietro *et al.*, 2024) are human skin commensal bacteria, but they can also become opportunistic and cause acne vulgaris (*P. acnes)* and sepsis or skin conditions like impetigo, depending on the species (*Streptococcus* spp.). Clade-specific patterns included *B. plebeius* (PC12) and *P. distasonis* (PC12) in Clade A, and *B. fragilis* (PC3 & PC9), *M. adhaesivum* (PC3 & PC4) in Clade C. *P. distasonis* (Ezeji *et al*., 2021; Cobo *et al.*, 2022) and *Bacteroides fragilis* (Wexler, 2007) are human commensals of the gut microbiome; however, they can be opportunistic. Both of these species are anaerobic bacteria that have been reported to be isolated from abscesses in humans and can cause bacteremia. However, *B. fragilis* is more well-studied for its medical importance as it is more common, especially in hospital settings, and only certain strains of *P. distasonis* have pathogenic potential. *P. distasonis* has also been reported previously in head lice (Abd Majid *et al.*, 2020). Similar to *B. fragilis, M. adhaesivum* may also cause healthcare-associated infection (Kovaleva *et al*., 2014) and an emerging opportunistic premise plumbing pathogen (Szwetkowski & Falkinham, 2020). Detection of *Staphylococcus aureus* (PC9) and *Staphylococcus* spp. (PC9 & PC4) in Clade C head lice is notable, given its established role in skin and systemic infections and its previous report in Malaysian head lice (Abd Majid *et al.*, 2020; Mokhtar *et al.*, 2020).

Other species, such as *A. cryaerophilus* in PC10_C (0.44%), *B. cereus* in PC10_C (0.10%), *P. nanceiensis* in PC1_C (0.35%), *Staphylococcus* spp. in PC4_C (0.21%) and PC9_C (0.01%), *V. parvula* in PC3_C (<0.01%) and PC10_C (0.08%), *H. parainfluenzae* in PC7_A (0.01%), *V. harveyi* in PC1_A (0.45%), *B. coprophilus* in PC7_A (0.03%) and PC12_A (0.02%) were detected at very low abundance (<1%), which may represent incidental carriage or background contamination. However, the presence of these bacteria, such as *Staphylococcus* spp., which is a significant opportunistic pathogen in skin diseases, and *H. parainfluenzae,* also an opportunistic pathogen, has been reported to cause meningitis and endocarditis (Black *et al.*, 1988). The detection of these bacteria should be taken into

consideration due to their medical danger on exposure to children. Overall, these findings echo global reports of head lice harbouring pathogenic bacteria (Amanzougaghene *et al.*, 2017), though none of the classic vector-borne agents (e.g., *Bartonella quintana*, *Coxiella burnetii*) were detected in this study.

These microbial findings intersect with the socio-sanitary and demographic factors identified earlier. Higher infestation prevalence among older children and females reflects behavioural and biological factors such as hair length and thickness, which may facilitate both head lice transmission and bacterial exposure. Environmental conditions, including reliance on river water and contact with stray animals, point to potential external microbial reservoirs. Especially the detection of microbes that have been associated with zoonotic pathogens and are derived from the environment. Eating with hands was independently associated with infestation risk, suggesting a behavioural pathway for ectoparasite transmission and possible microbial exposure. These results are consistent with previous Malaysian studies highlighting overcrowding, hygiene limitations, and communal practices as key risk factors for pediculosis (Lye *et al.*, 2017).

The co-occurrence of Clade A and Clade C within the same establishments (e.g., PC1 and PC9) suggests introduction through migration and inter-ethnic mixing, consistent with Malaysia's diverse shelter populations. Co-circulation of genetically distinct lineages may contribute to variation in microbiome composition. When coupled with socio-sanitary vulnerabilities, such diversity could increase the risk of opportunistic bacterial exposure among disadvantaged children.

While pooled 16S rRNA metagenomic sequencing provided an efficient and representative overview of bacterial composition across clades and establishments, this approach inherently limits resolution resolution at the individual louse level. Pooling masks within-group variability and prevents assessment of whether particular bacterial taxa are consistently associated with all head lice from a given host or site. Consequently, observed clade-dependent microbial patterns should be interpreted as population-level rather than individual level trends. Future studies employing more practical alternatives, such as targeted quantitative PCR or low-depth sequencing of selected individuals, may help validate pooled finding and clarify inter-individual variation.

Taken together, the integration of socio-demographic, genetic, and microbial data highlights the complex interplay between host factors, head lice clade distribution, and microbial carriage. The enrichment of opportunistic bacteria in Clade C, combined with higher infestation prevalence among children with specific behavioural and environmental exposures, underscores the dual public health burden of ectoparasite infestation and microbial risk in vulnerable populations. These findings support the need for integrated control strategies that combine pediculosis management with hygiene education and improved environmental conditions.

Conflict of interests

The author declares that they have no conflicts of interest.

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