

## Bacterial profiling of head lice isolated from the Orang Asli: A first report in Malaysia

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**Abstract.** This study was carried out to determine from bacterial profiling to the bacterial profiles of head lice among the Orang Asli communities. The head lice were collected from Orang Asli community volunteers. The surface sterilized head lice pools were subjected to genomic DNA extraction while next generation sequencing of the 16S rRNA gene was performed using the Illumina MiSeq platform. Six female and three male head lice identified as *Pediculus humanus capitis* were collected. A total of 111 368 number of NGS sequencing reads were recorded while another 223 bacterial taxa sequences were obtained. Symbiotic bacteria showed the highest number of reads, with *Arsenophonus* and *Rhodococcus* sequences being the most abundant genera in the female and male samples, respectively. The female head lice contained a more distinct microbial diversity. Amongst the pathogenic bacterial species sequences noted were the methicillin-resistant *Staphylococcus aureus*, *Streptobacillus moniliformis*, *Haemophilus influenzae*, *Bordetella pertussis* and *Acinetobacter baumannii*. The 16S rRNA genome sequencing revealed a number of rare and pathogenic bacterial species within the head lice of the Orang Asli. The socio-economic practices of the community which involved forest foraging and hunting, and their poor living conditions potentially facilitated the transmission of zoonotic bacterial pathogens, including those found within the head lice. Hence, there is the possibility that the head lice could serve as vectors for the transmission of pathogenic bacteria. This study highlighted the diverse microbial community found within the head lice's gut of the Orang Asli, with the detection of multiple rare and pathogenic bacteria capable of causing severe infections.

### INTRODUCTION

Globally, ectoparasitic infestations caused by head lice are an annoyance (Lesshafft *et al.*, 2013), especially to pre-school children and the poor communities of the developing world (Feldmeier, 2014). Humans can be infested by two lice species, a.) the human louse, *Pediculus humanus*, represented by two sub-species, the head louse (*Pediculus humanus capitis*) and the body louse (*Pediculus humanus humanus*); and b.) the pubic louse (*Phthirus pubis*) (Mumcuoglu, 2008).

The head louse is the most common and it is highly distributed with a prevalence of up to 61% throughout the world (Falagas *et al.*, 2008). The head louse is found on the human scalp and it feeds by sucking blood repeatedly. Female louse cements its eggs (nits) firmly to the base of the hair shaft after 5 to 9 days. Head louse infestation can cause intense scalp itchiness, dermatitis, and lymphadenitis due to the repeated inoculation of the louse saliva (Saghafipour *et al.*, 2017). The prolonged and chronic infestation by the head lice may in rare cases also cause anaemia, which is manifested by fatigue,

sleepiness as well as poor cognitive and learning function, especially among children (Bachok *et al.*, 2006).

To date, it is still unclear if the head lice can act as vectors of human pathogens. However, previous studies have reported the detection of *Bartonella quintana* DNA in head lice collected from Nepalese children (Sasaki *et al.*, 2006), homeless individuals from the USA (Bonilla *et al.*, 2009), Ethiopian patients (Angelakis *et al.*, 2011a), as well as nits collected from a homeless man in France (Angelakis *et al.*, 2011b). Previous studies on the prevalence of head lice in Malaysia focused on school-going children (Sinniah *et al.*, 1981; Bachok *et al.*, 2006; Muhammad Zayyid *et al.*, 2010), suggested an upsurge in the prevalence of head lice infestations among Malaysian children from 10.7% in 1981 (Sinniah *et al.*, 1981) to 35.0% in 2006 (Bachok *et al.*, 2006) and 49.0% in 2010 (Muhammad Zayyid *et al.*, 2010). To make matters worse, the resistance of head lice to pediculicides has been reported and is on the rise (Frankowski *et al.*, 2002). Most of the indigenous people or the Orang Asli of Malaysia that live within the forest fringe areas still follow the subsistence way of life, although some prefer to work in oil palm or rubber plantations (Masron *et al.*, 2013; Khor *et al.*, 2018). Their socio-economic practices and dependence on the forest expose them to the risk of contracting infectious diseases. These reasons are attributed to the fact that over 60% of the Orang Asli population in Kuala Milot to be infested with head lice (Vythilingam *et al.*, 1998). Aside from that and to the best of our knowledge, there has been no other study to look at the prevalence of head lice among the Orang Asli people.

Here, we sought to perform bacterial profiling of head lice isolated from the Orang Asli in Malaysia. The aim of the study was to identify potentially pathogenic bacterial species infecting this economically challenged population.

## MATERIALS AND METHODS

### **Ethics, consent, and permission for sample collection**

This study was conducted in April 2017 in an Orang Asli settlement (101.917726°E, 3.143844°N), located in the state of Selangor, Malaysia. Sample collections were performed with approval from the Ethics Committee of the University Malaya Medical Centre, Malaysia (MREC: 20161115-4602) and the Department of Orang Asli Development, Malaysia (JAKOA). Prior to sample collection, questionnaires and written consent forms were distributed to each participant and sampling was only performed after receiving consent. Consent was also obtained from respective parents or guardians on behalf of their children who are under the age of 18.

A fine-tooth comb was used to remove the head lice from the volunteer's hair. At the time of sampling, three female volunteers from 30 participants were afflicted with the head lice. The samples were kept in liquid nitrogen and transported to the Tropical Infectious Diseases Research & Education Centre (TIDREC), University of Malaya, followed by storage in -80°C.

### **Identification of head lice samples**

The collected head lice were observed under the microscope to identify the species and further classify the life stages based on the published taxonomic keys for *Pediculus* lice (Bonilla *et al.*, 2013).

### **DNA extraction**

The head lice were pooled according to their gender and surface sterilized strictly following the established protocols (Lim *et al.*, 2017). Briefly, pooled samples were pulverized and powdered in liquid nitrogen using chilled mortar and pestle. The powdered samples were then suspended with sterile phosphate-buffered saline followed by

DNA extraction using the NucleoSpin Tissue kit (Macherey-Nagel, Duren, Germany). The extracted genomic DNA from the male and female head lice pools were sent to a third-party service provider (Biolution Resources, Selangor, Malaysia) for metagenomics analysis using the Illumina MiSeq platform.

### Data and sequence analysis

The generated raw reads were stored in fastq format, followed by demultiplexing and quality-filtering using QIIME (version 1.9.1). The paired-reads were merged into a single read and subjected to operational taxonomic unit (OTU) clustering, with 97% similarity cut off using UPARSE (<http://drive5.com/uparse>). In parallel, chimeric sequences were identified and removed using UCHIME (<http://www.drive5.com/uchime/>). The taxonomy of each sequence was analysed by RDP Classifier (<http://rdp.cme.msu.edu/>) against the SILVA 16S rRNA database (<http://www.arb-silva.de>) using the confidence threshold of 0.7. The rarefaction curves were plotted to assess species richness in the respective samples using mothur (<https://www.mothur.org/>). The bacterial community composition in the samples was computed using R programming language (<http://www.r-project.org/>).

### Phylogenetic tree analysis of selected bacterial taxa

Selected bacterial genera or species were compared for similarity with other pathogenic strains and their phylogenetic relationships inferred using the neighbour-joining method implemented in MEGA (version 6.0). The phylogenetic tree was tested using the Kimura-2 parameter algorithm with 1000 bootstrap replicates.

## RESULTS

### Head lice samples

Nine adult head lice were identified as *Pediculus humanus capitis*, comprising of six females and three males. They were collected from three female children from Orang Asli volunteers from a total of 50 volunteers (adults and children).

### 16S rRNA sequencing results

The amplicons for the bacterial 16S rRNA gene of the pooled male sample yielded 53 232 number of reads, while the pooled female sample yielded 58 136 number of reads for downstream analysis. A total of 53 655 (around 421–440bp) and 59 259 (around 441–460bp) valid sequences were generated after assembly and trimming using the Trimmomatic and FLASH softwares (Majorbio, China). The samples were rarefied for OTU clustering and taxonomic assignment for the subsequent bacterial diversity analysis.

### Bacterial community detected in head louse samples

The rarefaction curves for the male pool approached saturation at 53 200 reads while the female reported 57 800 reads, detecting a total of 223 taxa. At the class level, six major classes were observed, namely, *Actinobacteria* (31/223, taxa/total taxa), *Alphaproteobacteria* (20/223), *Bacteroidia* (26/223), *Bacilli* (16/223), *Betaproteobacteria* (16/223), and *Gammaproteobacteria* (21/223). Only 14 bacterial taxa were most represented ( $\geq 0.5\%$ ) for both pools combined (93.96%), in which 12 out of 14 bacterial taxa were assigned to the genus level (90.59%) (Table 1). A total of 145 bacterial taxa were found in the female pool while 163 taxa were identified in the male pool.

Out of the total 111 368 number of reads, *Rhodococcus* was the most abundant genus found in both the male and female pools (31.85%), followed by *Arsenophonus* (20.91%), *Staphylococcus* (13.96%), *Microbacterium* (9.75%), *Haemophilus* (6.59%), *Escherichia-Shigella* (1.94%), *Streptococcus* (1.65%), *Pseudomonas* (1.37%), *Bordetella* (0.76%), *Propionibacterium* (0.72%), *Achromobacter* (0.56%), and *Bacteroides* (0.53%) (Table 1). *Arsenophonus* (37.35%) and *Rhodococcus* (36.74%) were the most dominant genera identified in the female and male pools, respectively (Figure 1). Meanwhile, 0.08% and 0.47% of the reads in the respective female and male pools, were not assigned to any genus.

Table 1. Assigned taxa with abundance > 0.5% of total population in head lice samples

Assigned taxa	Abundance (%)
d__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Corynebacteriales; f__Nocardiaceae; g__Rhodococcus	31.85
d__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Arsenophonus	20.91
d__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Staphylococcaceae; g__Staphylococcus	13.96
d__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Micrococcales; f__Microbacteriaceae; g__Microbacterium	9.75
d__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pasteurellales; f__Pasteurellaceae; g__Haemophilus	6.59
d__Bacteria; p__Firmicutes; c__Clostridia; o__ Clostridiales; f__Other; g__Other	2.23
d__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Enterobacteriales; f__Enterobacteriaceae; g__Escherichia-Shigella	1.94
d__Bacteria; p__Firmicutes; c__Bacilli; o__Lactobacillales; f__Streptococcaceae; g__Streptococcus	1.65
d__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Pseudomonadales; f__Pseudomonadaceae; g__Pseudomonas	1.37
d__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Other; f__Other; g__Other	1.14
d__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Bordetella	0.76
d__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Propionibacteriales; f__Propionibacteriaceae; g__Propionibacterium	0.72
d__Bacteria; p__Proteobacteria; c__Betaproteobacteria; o__Burkholderiales; f__Alcaligenaceae; g__Achromobacter	0.56
d__Bacteria; p__Bacteroidetes; c__Bacteroidia; o__Bacteroidales; f__Bacteroidaceae; g__Bacteroides	0.53

\*d: domain; p: phylum; c: class; o: order; f: family; g: genus; >: greater than; %: percentage.

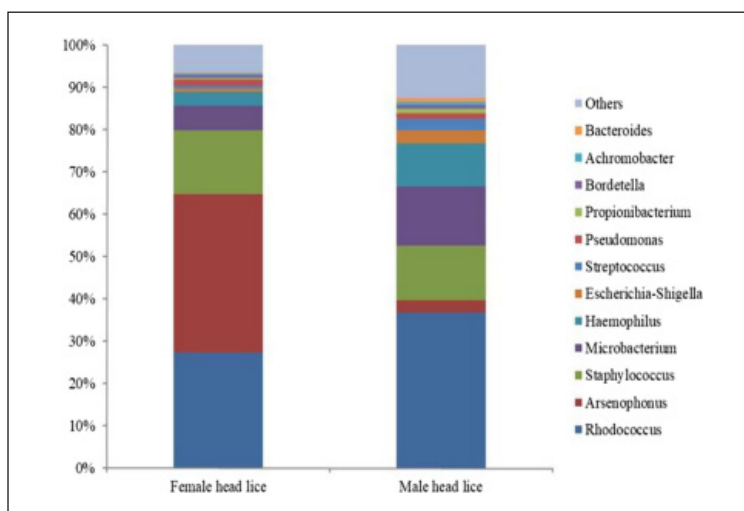


Figure 1. Relative abundance of bacterial populations at the genus level in head lice samples.

The detected bacterial taxa in the head lice samples were broadly separated into three groups comprising of the endosymbionts (*Candidatus Riesia pediculicola* and *Rhodococcus*), the medically-important/potentially pathogenic bacteria (*Streptococcus salivarius*, *S. anginosus*, *Staphylococcus aureus*, *S. sciuri*, *Streptobacillus moniliformis*, *Propionibacterium acnes*, *Citrobacter koseri*, *Bergeyella zoohelcum*, *Ralstonia pickettii*, *Bordetella pertussis*, *Empedobacter brevis*, *Parabacteroids distasonis*, *Haemophilus influenzae* and *Acinetobacter baumannii*) and the environmental bacteria (*Bacillus*, *Methylobacterium*, and *Sphingomonas*) (Table 2). *S. aureus* was the most prevalent bacterial species observed in both female and male pools, and potentially pathogenic bacteria was more abundantly found in the male lice.

### Phylogenetic tree of selected pathogenic bacterial species

The 16S rRNA sequence analysis between the medically-important/potentially pathogenic bacterial species found in this study and other clinical isolates obtained from the GenBank was shown in Figure 2. From the phylogenetic tree, OTU20 and OTU169 were clustered into the streptococci clade together with *S. anginosus* (NR041722) and *S. salivarius* (AF459433), while the staphylococci clade gathered OTU41, OTU52, *S. aureus* (AM980864) and *S. sciuri* (AB662960). OTU64, OTU 125 and OTU143 were grouped with *Bergeyella* sp. (AY289204 and AY398696), *E. brevis* (NR042471) and *P. distasonis* (AB238922 and AB238924), possibly reflecting their origin from the bacterial phylum *Bacteroidetes*. OTU67 and OTU111 were clustered together with *B.*

Table 2. Relative abundance of different types of bacteria isolated from female and male head lice

OTU ID	Bacterial taxon	Relative abundance (%)	
		Female	Male
Endosymbiont bacteria			
OTU117	<i>Candidatus Riesia pediculicola</i>	37.3	2.96
OTU173	<i>Rhodococcus</i> sp.	30	36
Medically-important/pathogenic bacteria			
OTU20	<i>Streptococcus salivarius</i>	0.12	0.2
OTU41	<i>Staphylococcus aureus</i> MRSA	15	12.77
OTU42	<i>Streptobacillus moniliformis</i>	0	0.39
OTU52	<i>Staphylococcus sciuri</i> subsp. <i>sciuri</i>	0.05	0
OTU53	<i>Propionibacterium acnes</i>	0.33	1.12
OTU58	<i>Citrobacter koseri</i>	0.007	0.22
OTU64	<i>Bergeyella zoohelcum</i>	0	0.33
OTU67	<i>Ralstonia pickettii</i>	0.03	0
OTU111	<i>Bordetella pertussis</i>	0.64	0.9
OTU125	<i>Empedobacter brevis</i>	0.11	0
OTU143	<i>Parabacteroides distasonis</i>	0.03	0
OTU161	<i>Haemophilus influenzae</i>	3.32	10.16
OTU167	<i>Acinetobacter baumannii</i>	0.06	0.008
OTU169	<i>Streptococcus anginosus</i> subsp. <i>anginosus</i>	0	0.05
Environmental bacteria			
OTU127	<i>Bacillus</i> sp.	0	0.024
OTU5	<i>Methylobacterium</i> sp.	0.366	0
OTU27	<i>Sphingomonas echinoides</i>	0.179	0.036
OTU103	<i>Sphingomonas kaistensis</i>	0	0.085
OTU153	<i>Sphingomonas aerolata</i>	0.05	0

\*OTU: Operational Taxonomic Unit; %: percentage.

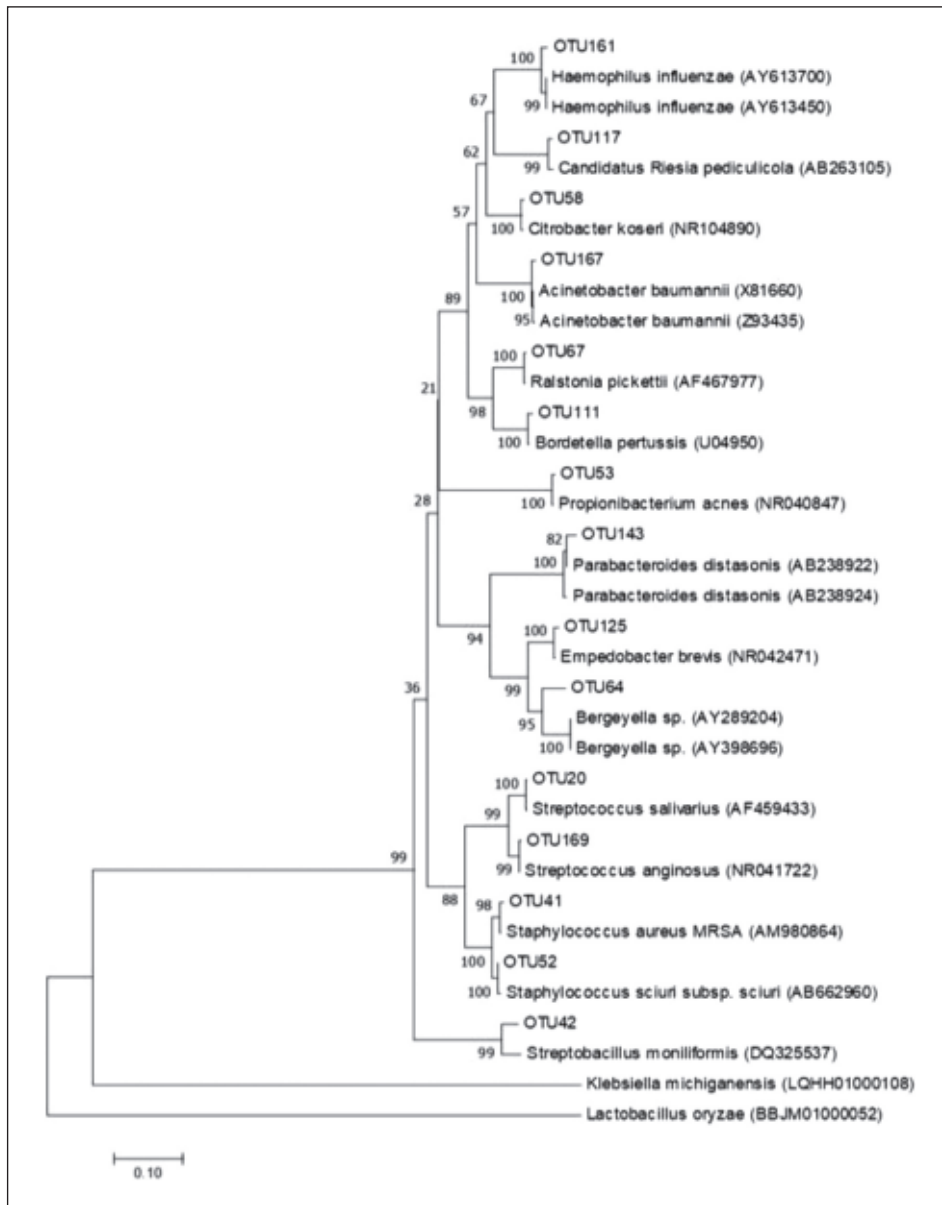


Figure 2. Phylogenetic tree derived from 16S rRNA bacteria sequences isolated from head lice in the present study compared to other bacterial isolates available from GenBank. The tree was calculated using neighbor-joining method. The numbers on branch points depict the values of 1000 bootstrap replicates. GenBank accession numbers for reference sequences are indicated at the end of each species designations.

*pertussis* (U04950) and *R. pickettii* (AF467977), suggesting that they belonged to the class  $\beta$ -proteobacteria. OTU58, OTU161 and OTU167 likely belonged to the class  $\gamma$ -proteobacteria as they were grouped with *A. baumannii* (X81660 and

Z93435), *C. koseri* (NR104890) and *H. influenzae* (AY613700 and AY13450). OTU117 and OTU53 were clustered respectively with *C. R. pediculicola* (AB263105) and *P. acnes* (NR040847).

## DISCUSSION

The indigenous communities living in forest-fringe areas, located away from modern healthcare infrastructures are often economically marginalised (Masron *et al.*, 2013; Khor *et al.*, 2018). Numerous initiatives to improve the living standards of the Orang Asli communities had been carried out by the Malaysian government over the years, including the continuous education and awareness campaigns on hygiene practices (Chandren *et al.*, 2015). Despite all these efforts, head lice infestation remains a problem. Vythilingam *et al.* (1998) reported the only head lice prevalence study in an Orang Asli village in Pahang, Malaysia, however, they did not document the diversity of potentially pathogenic bacterial species found within the head lice, hence, could not ascertain if head lice could serve as vector for transmission of pathogens.

Arthropods are colonised by numerous microorganisms, amongst them bacteria that share symbiotic relationships important for the host nutrition, growth, and pathogen defence (Chen *et al.*, 2016). Head lice infestation among humans is commonly associated with people from the lower socioeconomic groups (Falagas *et al.*, 2008) with children being frequently affected (Meister & Ochsendorf, 2016). Similar incidences had also been monitored among the Orang Asli children in Perak with 46.3% being infested with head lice. Based on the report, girls (74.7%) showed a higher prevalence compared to boys (13.2%) (Soe *et al.*, 2018).

The 16S gene was used for the investigation of bacterial diversity in the head lice because it has been successfully utilised for the detection of rare and pathogenic bacterial species (Schrottner *et al.*, 2016). Given that the head lice have a restricted hematophagous diet (Rodríguez *et al.*, 2011), they could host endosymbionts to provide them with vitamins and other nutrients essential for development and growth. It was not surprising, therefore, to find relatively high numbers of the genera *Rhodococcus* (Rodríguez *et al.*, 2011), *Arsenophonus* (Šochová *et al.*, 2017), and *C.*

*R. pediculicola* (Allen *et al.*, 2007), which are known head lice endosymbionts (Tables 1 and 2). The socio-economic status and daily routine activities of the Orang Asli which involves forest foraging, very likely exposes them to ubiquitous environmental bacteria such as the *Bacillus* sp. (Diamoande *et al.*, 2015), *Methylobacterium* sp. (Gallego *et al.*, 2005), and *Sphingomonas* sp. (Leys *et al.*, 2004), encountered in this study, albeit at very low relative abundances (Table 2).

*Acinetobacter* sp. stood out as the most commonly detected pathogenic bacteria in the human head lice, besides *B. quintana* and *B. recurrentis* (Mana *et al.*, 2017). However, this study revealed a higher presence of other pathogenic bacteria (in terms of relative abundance) that had not been widely reported to be associated with human head lice. Our findings detected more female head lice infestations, similar to a previous study (Perotti *et al.*, 2004). Nonetheless, pathogenic bacterial species was found relatively higher in the male (26.148%) compared to female pools (19.697%). Although several questions arise on the gaps of pathogenic density between male and female head lice, both sexes could potentially harbour a number of pathogens detrimental to human health.

Staphylococci represented the highest medically-important bacteria genera detected in the head lice samples (Table 1 and Figure 1), particularly methicillin-resistant *S. aureus* (MRSA). Abrasions of the scalp due to head lice infestation could lead to soft tissue infections by the MRSA (Sam *et al.*, 2008). The acquisition of *S. sciuri* by head lice could possibly reflect transmission from animals to the Orang Asli as this bacterium is usually an animal skin colonizer (Beims *et al.*, 2016). Likewise, *S. moniliformis* (Table 2) and *B. zoohelcum* (Figure 2) were most likely to have originated from the wild rats (Suzuki *et al.*, 2017) and pets (Chen *et al.*, 2017), respectively. It is reasonable to suggest that these transmissions are due to frequent contacts with domesticated or wild animals that are consistent with the socio-economic practices of the Orang Asli.

The next highest relative microbial abundance found in the head lice samples belonged to the genera *Microbacterium* and *Haemophilus* (Table 1 and Figure 1). Members of both genera have been implicated in clinical cases with *M. paraoxydans* causing dialysis-related peritonitis (Choi *et al.*, 2017) and *Haemophilus influenzae*, the leading cause of meningitis, epiglottitis, and pneumonia in children under five years old (Monge *et al.*, 2018). This pathogen is covered in the national vaccination program. *Bordetella pertussis*, the agent of whooping cough, found in the studied head lice samples, is also covered by the Malaysian vaccination program (Wong *et al.*, 2008). These examples illustrate the risk of infectious diseases transmission to the Orang Asli, as the pathogenic bacteria exist in relatively high abundance in the collected head lice.

Apart from that, sequencing of the collected head lice detected a number of opportunistic pathogens, supposedly existing as commensal bacteria in many mammals. *P. acnes* (Figure 2), primarily found on the mammalian skin, has been associated with invasive and nosocomial infections (Achermann *et al.*, 2014). The head lice could have acquired this bacterium from the scalp of the Orang Asli. Similarly, *S. salivarius* (Delorme *et al.*, 2011), *S. anginosus* (Erdem *et al.*, 2015), *C. koseri* (Dzeing-Ella *et al.*, 2009), *R. pickettii* (Stelzmueller *et al.*, 2006), and *P. distasonis* (Kierzkowska *et al.*, 2017) (Figure 2) are commensals of the oral cavity and gastrointestinal tracts, and they have been isolated and implicated in human clinical cases, some leading to deaths. The detection of *E. brevis* (Table 2 and Figure 2), an environmental inhabitant occasionally found in clinical specimens, underlines the potential threat of obscure pathogens on humans, especially so when the pathogen is adept at surviving in the hospital environment (Sharma *et al.*, 2016). Although there is still a lack of conclusive evidence to resolve the controversial role of head lice as disease vectors, we found substantial genomic evidence pointing to the existence of a large pathogenic microbial community within the head lice. The possibility of head lice

transmitting pathogenic bacteria originating from a contaminated blood meal (Boutellis *et al.*, 2013) can prove to be a challenging public health issue.

Despite the thorough surface sterilization process being carried out, diverse numbers of bacteria were found on the surface of head lice samples, that either originated from the environment or as part of the mammalian skin flora. This similar process had been previously adopted (Carpi *et al.*, 2011; Menchaca *et al.*, 2013; Khoo *et al.*, 2016), describing the presence of bacterial communities on the exoskeletons. Therefore, the washing step is deemed necessary to eliminate contaminants from the surroundings (Carpi *et al.*, 2011), with more vigorous procedures incorporated for future studies (Khoo *et al.*, 2016). This is still a limitation as surface sterilization does not remove DNA from the specimen, therefore, normal flora detected in the findings may still be the residual DNA from the host.

The isolation of a relatively small number of head lice from a single site in this study represented another limitation, although interestingly, this could be due to the use of *Pongamia pinnata* leaf extracts, a traditional medicine of the Orang Asli, to control pediculosis (Anbu *et al.*, 2009). In order to overcome this limitation, more Orang Asli settlements from a wider area can be included, hence, a better representation of the microbial community within the head lice can be obtained. The diversity of bacteria detected in this study showed the propensity of the head lice to act as a host for rare pathogenic bacterial species, although its role as a vector has yet to be demonstrated. Nevertheless, our findings outline the baseline data for the risk and potential hazard of the transmission of infectious diseases from head lice from this community. There is also an urgent need to make an inventory of the list of bacterial communities within the head lice and study its association with pediculosis. In addition, continuous education and awareness programs are vital in improving the hygiene practices among the Orang Asli, crucial in eradicating head lice infestations.



## CONCLUSION

This study highlights the bacterial diversity in the gut of head lice, with the detection of rare and pathogenic bacteria capable of causing harmful infections. Some of the detected bacterial species have an animal origin, suggesting the potential transfer of zoonotic diseases to the Orang Asli.

### Conflict of interest

The authors declare that they have no conflict of interest.

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