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RESEARCH ARTICLE

Identification of skin bacterial profiles of early deceased bodies and the relation to post-mortem interval

Chong, C.K.[1], Emamjomeh, M.[1], Joseph, N.[1], Siew, S.F.[2], Maeda, T.[3], Mustapha, N.A.[3], Hoshiko, Y.[4], Muthanna, A.[5], Amin-Nordin, S.[1*]

[1]Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia
[2]Department of Forensic Medicine, Kuala Lumpur Hospital, 50586 Federal Territory of Kuala Lumpur, Malaysia
[3]Department of Biological Functions Engineering, Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu-ku, Kitakyushu 808-0196 Japan
[4]Department of Infectious Medicine, Kurume University School of Medicine, 67 Asahi-Machi, Kurume-City, Fukuoka 830-0011 Japan
[5]Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor Darul Ehsan, Malaysia
*Corresponding author: syafinaz@upm.edu.my

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ABSTRACT

Post-mortem microbiology (PMM) is an important tool in identifying possible causes of sudden unexpected death, as an infectious cause is highly suspected. However, contamination is a major problem in microbiology, and this has increased the difficulty determining the true pathogen that contributes to death in post-mortem cases. Skin commensals are common contaminants in blood cultures. This study was conducted to investigate the skin flora on early deceased bodies and observe the bacteria detected at different post-mortem intervals (PMIs). As blood is usually drawn from the neck and femoral sites for PMM examination, the two body sites were chosen as the sampling sites. Skin swab samples from the neck and femoral (n=80) of each early deceased body were collected by sterile cotton swabs. DNA was extracted from the swabs and then subjected to high-throughput 16S rRNA sequencing by using the Illumina MiSeq platform. *Staphylococcus* was found to be the most dominant genus in both neck and femoral sites. LEfSe results showed that *Cutibacterium* is significantly different at the neck site while *Corynebacterium* is more abundant at femoral site. There are significant differences at genus level between PMI<5H and PMI>5H at both neck and femoral sites. The findings of the present study may act as a reference for microbiologists and forensic pathologists when mixed growth or contamination occurs in post-mortem blood cultures.

Keywords: Post-mortem microbiology; sudden unexpected death; post-mortem interval; skin flora; high-throughput 16S rRNA sequencing.

INTRODUCTION

Post-mortem microbiology (PMM) is relatively important in the field of forensic pathology. The result from PMM examination is valuable in determining the cause of death, especially caused by infection. PMM is a standard protocol in sudden unexpected death cases at any age (Palmiere *et al*., 2016).

PMM identifies unknown pathogens or biological warfare agents that aid in the prevention of bioterrorism (Bhatia *et al*., 2016). PMM has the potential to prevent the occurrence of outbreaks that are harmful to people given the ability to determine the pathogenic agents. PMM is useful in evaluating ante-mortem clinical findings. In addition, PMM can compare post-mortem findings and ante-mortem clinical reports to evaluate the efficacy of treatment, especially for antimicrobial agents (Riedel, 2014). As the bacterial community changes during the decomposition process, PMM is considered an important tool in determining the post-mortem interval (PMI) of the body (Metcalf *et al*., 2013, 2016, 2017; Pechal *et al*., 2014; Finley *et al*., 2015; Hyde *et al*., 2015; Javan *et al*., 2016; Johnson *et al*., 2016; Oliveira & Amorim, 2018; Dong *et al*., 2019). PMI is particularly necessary in criminal cases to track down the suspect.

PMM results have been distinguished into a few categories, which are truly positive, false positive results, including agonal spread, bacterial translocation, and contamination, as well as negative results (Riedel, 2014; Christoffersen, 2015; Na *et al*., 2016). Truly positive means the isolation of the true pathogen that could possibly cause the death, which is usually one isolate. False positive results usually isolate more than two organisms (Palmiere *et al*., 2016; Ventura Spagnolo *et al*., 2019; Saegeman *et al*., 2021).

Contamination is a commonly occurring problem. The bacteria are introduced into the sample at the moment they are obtained (Morris *et al*., 2007; Christoffersen, 2015; Na *et al*., 2016; Palmiere *et al*., 2016; Saegeman *et al*., 2021). Contamination usually isolates more than two organisms and skin commensals are the common contaminants in blood samples. Nevertheless, the differentiation between contamination and true infection remains a major challenge to forensic pathologists and clinical microbiologists in determining the possible cause of death. Contamination has caused the difficulty in interpretation of PMM results.

PMM examination is necessary for natural death cases. However, the contamination of the samples may increase the difficulty in interpretation of possible cause of death, especially caused by infection. Hospital Kuala Lumpur in Malaysia has suffered from contamination of PMM samples for years. When mixed growth occurs, clinical microbiologists and forensic pathologists are unable to identify the true pathogen because the true pathogen may be covered or inhibited by other bacteria. Skin flora on early deceased bodies appear critical in dealing with this problem. Sampling site and the relevant skin flora are recommended to take into account for the interpretation of PMM examination results, as stated in the study of (Khalid *et al*., 2018). As we know that *Corynebacterium*, *Propionibacterium*, *Staphylococcus* are well known skin commensals bacteria (Grice & Segre, 2011). By knowing the normal skin flora on early deceased bodies, they can act as a reference for microbiologists and forensic pathologists for natural death cases when contamination happens. This situation allows an increase in the accuracy of the interpretation of PMM results.

This study aims to investigate the skin flora on early deceased bodies at the neck and femoral sites, as these two sites are usually taken for blood samples for the PMM examination in Malaysia. This study also aims to compare the differences in bacterial profiles collected from neck and femoral sites. In addition, the dynamic pattern of the flora profiles in relation to PMI is investigated.

MATERIALS AND METHODS

Study design

Inclusion criteria for the remains included adults with natural causes of death and their duration of the earliest documented time of death to sampling time, which was known as the estimated post-mortem interval. The estimated post-mortem interval (PMI) in this article was within 24 hours. The time of death of the deceased, which was stated by witnesses or police officers, or announced by paramedics, was recorded. Patients with skin diseases, iatrogenic marks, or surgical intervention in the areas of interest were excluded. The deceased bodies were collected at the forensic department in Hospital Kuala Lumpur, Malaysia. This study was approved by the Malaysian Medical Research and Ethics Committee (Ref No. NMRR-19-2026-48509) and the Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (Ref No. JKEUPM-2020-419). A total of 40 bodies (aged 27- 69 years old), with the PMI ranged from 0.25 h to 17.27 h were included in this study. The summary of the age, gender, PMI, and the cause of death for the deceased samples were shown in Table 1. Written consent was given to all next-of-kin of participants, and they received written information about the study before sample collection.

Table 1. Summary of the age, gender, estimated post-mortem interval (PMI) and cause of death of the deceased samples

Sample No.	Age	Gender	Estimated Postmortem Interval (PMI)	Cause of Death
1	39	Female	17.27	Undertermined
2	27	Male	1.92	Undertermined
3	57	Male	3.67	Heart related disease
4	69	Male	19	Heart related disease
5	30	Male	4.83	Heart related disease
6	40	Male	3.5	Heart related disease
7	53	Male	4	Heart related disease
8	40	Male	2.5	Infection
9	32	Male	10.67	Heart related disease
10	40	Male	2.5	Others
11	39	Male	2.33	Heart related disease
12	43	Male	3.08	Heart related disease
13	31	Female	5.82	Others
14	48	Male	0.5	Heart related disease
15	55	Male	0.33	Heart related disease
16	30	Male	6.65	Heart related disease
17	30	Female	2.83	Infection
18	56	Male	4.5	Heart related disease
19	43	Male	1.63	Undertermined
20	63	Male	4.13	Infection
21	57	Male	0.67	Heart related disease
22	56	Male	1.72	Others
23	58	Male	4.25	Heart related disease
24	30	Female	1.57	Others
25	52	Female	6.33	Heart related disease
26	43	Male	2.4	Heart related disease
27	48	Male	2	Heart related disease
28	60	Male	2.92	Heart related disease
29	49	Male	1.12	Heart related disease
30	42	Male	3.67	Others
31	60~70	Male	2.42	Others
32	35	Male	1.65	Undertermined
33	65	Male	1.42	Heart related disease
34	59	Male	3.15	Heart related disease
35	40	Female	2.58	Heart related disease
36	60	Female	1.83	Heart related disease
37	53	Male	0.67	Heart related disease
38	36	Male	5.6	Heart related disease
39	45	Male	3	Heart related disease
40	65	Female	0.25	Heart related disease

Sample collection

Two body sites, neck and femoral, from each body were used for skin samples. As blood culture is usually taken from these neck and femoral sites in PMM examination, skin swabs from these two sites were chosen as samples. The skin swabs samples were taken prior to blood culture collections. The bodies were checked after arriving at the hospital to determine if they fulfilled the inclusion and exclusion criteria of the study. Subsequently, the skin swab samples were taken prior to the body's storage in the freezer which is between 2°C to 8°C. Neither sterilisation nor cleaning of the body was done for taking skin samples. Samples were collected using sterile cotton swabs moistened with normal saline. The front right site, around 4×4cm of the neck site was taken as samples while the femoral site was taken samples from femoral triangle. The borders of femoral triangle were composed by sartorius muscle as the lateral border, adductor longus muscle as the medial border, and inguinal ligament as the superior border. The swabs were rubbed vigorously and rotated to ensure homogeneity in the skin areas contacted by each swab, around 15-30 times (Fierer *et al*., 2010; Myles *et al*., 2016). The collection procedure was performed by a trained researcher. The swabs were then transported in 200 µL of phosphate-buffered saline (PBS), which acted as transport media in a sterile 15 mL centrifuge tube for storage. The samples were transported to the laboratory in a container at room temperature within 24 hours and stored in a freezer (-20°C).

DNA extraction and polymerase chain reaction (PCR)

DNA extraction was done by using QIAamp® DNA Microbiome Kit (Qiagen, Germany) following manufacturer instructions with some optimisations. Sample was first pre-treated by immersing the swab sample in 1 mL PBS before extracted. Double elution was performed to ensure a higher yield of microbial DNA. The eluate was used to repeated the elution step according to the manufacturer instruction. Concentration of extracted DNA was quantified by NanoDrop Spectrophotometer (Thermo Fisher Scientific, Massachusetts, U.S). The extracted DNA was stored at -20°C before conducting further amplification processes. Primers 341F (5'-CCTACGGGNGGCWGCAG -3') and 785R (5'-GACTACHVGGGTATCTAATCC-3') were selected to amplify the 16S rRNA gene of the extracted DNA (Klindworth *et al*., 2013). Five microliters of DNA extracted were used for a 25 µL PCR reaction, containing master mix (Qiagen, Germany), forward and reverse primers, nuclease-free water and DNA template. Thermal cycling was done under the following conditions: initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min. After that, the final extension was detected at 72°C for 10 min, and ended with a final cooling step at 4°C. The amplified PCR products were verified by running agarose gel electrophoresis. A 1.7% agarose gel was used. A total of 60 min of electrophoresis was run at 80 V. The gel was finally examined by using the Gel Documenting System (Bio-Rad Laboratories, Inc, US).

High throughput 16S rRNA sequencing

The forward and reverse primers, 341F (5'-CCTACGGGNGGCWGCAG-3') and 785R (5'-GACTACHVGGGTATCTAATCC-3'), that targeted V3-V4 hypervariable region of the 16S rRNA gene was used to amplify the extracted DNA samples (Klindworth *et al*., 2013). The amplification and library preparation were conducted according to the 16S metagenomics sequencing library preparation protocol for Illumina MiSeq system (Part # 15044223 Rev. B). For samples with low concentration of DNA, the first round of PCR amplification was slightly modified. The PCR mixture consisted of 10× Taq buffer and Taq polymerase (Biolabs, USA), 1 µM of each primer, 2 mM each dNTPs mix, 25 mM of $MgSO_4$ (Toyobo, Japan), and 2 µL of DNA template. PCR amplification was carried out under following conditions: initial denaturation 94°C for 45 s, 50°C for 60 s, and 72°C for 90 s of 35 cycles, and a final extension at 72°C for 10 min. The rest of library preparation steps were conducted according to the 16S metagenomics sequencing library preparation for Illumina MiSeq as described previously (Mustapha *et al*., 2018). In brief, the amplification of DNA started with amplicon PCR for 25 cycles. The PCR product was then purified by using AMPure XP beads, followed by Index PCR to attach dual indices and Illumina sequencing adapters by using the Nextera XT Index Kit (Illumina Inc., USA). Purification of PCR products was repeated prior to library quantification. Then, the library was normalised to ensure that the pooled samples generated a representative library. Pooled samples were denatured by NaOH and heat-denatured prior to sequencing. PhiX with 30% spike-in was added as an internal control for the sequencing. The combined pooled samples and PhiX were then loaded into a 600-cycle V3 MiSeq reagent cartridge (Illumina), which was then attached to the Illumina MiSeq Sequencing System along with a flow cell. Sequencing was performed for 301, 8, 8, and 301 cycles for forward, index 1, index 2, and reverse reads, respectively. Demultiplexed raw data were obtained and analysed. The raw sequence data were deposited into the National Centre for Biotechnology Information (NCBI) short read archive database under the accession number of SRP339843.

Data processing and analysis

High throughput data were processed using QIIME 2 2021.11 (Bolyen *et al*., 2019). Delmultiplexed raw data was denoised by using cutadapt and DADA2 (Callahan *et al*., 2016). A phylogenetic tree was generated by FastTree (Price *et al*., 2010). A naive Bayes feature classifier was trained on the SILVA-138-99 database (Quast *et al*., 2013) using the q2-feature-classifier plugin (Bokulich *et al*., 2018), and taxonomy was assigned using the q2-classify-sklearn plugin (Pedregosa *et al*., 2011). The assembled sequences were then clustered into Operational Taxonomic Unit (OTU) at a cut-off of 97% sequence identities. Samples were rarefied at a sampling depth of 5900 sequences for diversity analyses which has caused three samples excluded from the analysis (1pN and 34pN, two samples from neck site, and 40pF, one sample from femoral site). Alpha diversity indices, such as Shannon diversity, observed features, faith-phylogenetic diversity, evenness, and beta diversity index (weighted UniFrac distance) were generated. Dominant genera in both neck and femoral sites as well as in both PMI groups were determined using relative abundance. Differential abundance was analysed by using linear discriminant analysis effect size (LEfSe) (Segata *et al*., 2011) to identify the significant difference of taxa abundance between PMI groups and body sites which are neck and femoral sites at genus level (Kruskal-Wallis test, p-value < 0.05, LDA score > 2.0).

RESULTS

Microbiological profile of bacteria at neck and femoral sites

Alpha Diversity. The figure showed there is no significant difference between femoral and neck sites in Pielou's evenness (Figure 1a, p=0.06), phylogenetic diversity (Figure 1b, p=0.88), observed features (Figure 1c, p=0.93), and Shannon index (Figure 1d, p=0.23) at a sample depth of 5900.

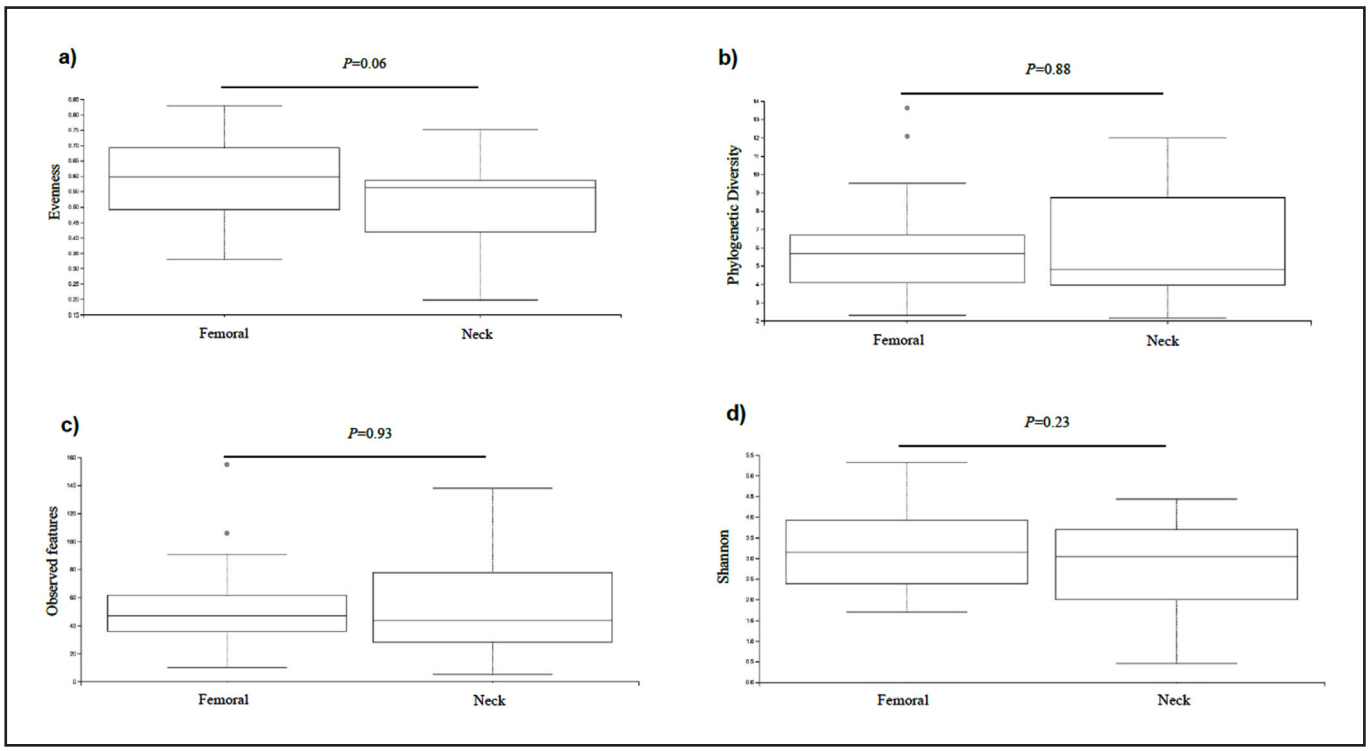

Figure 1. The comparison of alpha diversity indices which are Pielou's evenness (a), phylogenetic diversity (b), observed features (c) and Shannon index (d) between the neck and femoral sites at sample depth of 5900.

Beta Diversity. Principal Coordinate Analysis (PCoA) based on weighted UniFrac distance (Figure 2) illustrated clustering of samples by sampling sites, which were the femoral and neck sites. The PERMANOVA analysis for weighted UniFrac distance matrix showed that the bacterial composition was detected at the neck and femoral had no significant difference (p-value=0.10).

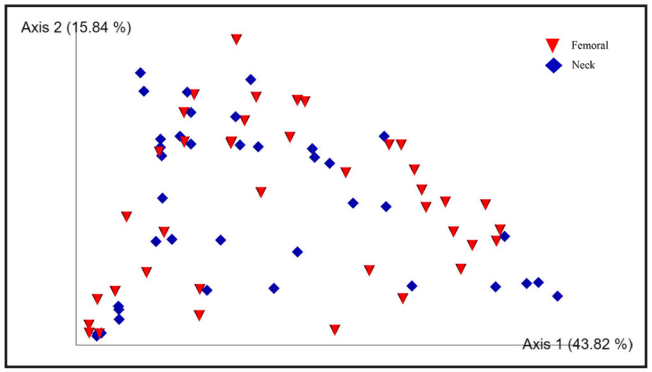

Figure 2. Two-dimensional PCoA plot based on weighted UniFrac distance showed no significant difference between femoral and neck sites. Red, Femoral site (*n*=39); Blue, Neck site (*n*=38).

Relative Abundance. The dominant phylotypes in the samples belonged to the phylum *Proteobacteria* (57.28-66.50% of total reads), followed by *Firmicutes* (24.38-26.35%), *Actinobacteria* (6.83-14.78%) and *Bacteroidota* (0.35-1.28%). The top 10 dominant genera that were detected in both the neck and femoral sites were visualised in bar chart form (Figure 3). *Staphylococcus* was the most dominant genus found in both femoral and neck sites (Femoral, 24.38%; Neck, 22.75%). At the femoral site, *Corynebacterium* appeared to be abundant (14.43%), which ranked the second dominant genus in the list, followed by various members of *Proteobacteria*. For the neck site, after the most abundant genus, *Staphylococcus* was found, followed by members of *Proteobacteria* which are unclassified *Enterobacteriaceae* (16.95%), unclassified *Rhizobiaceae* (14.47%), *Ochrobactrum* (14.22%), and more. The majority of the genera were *Proteobacteria* members at both body sites. LEfSe (Figure 4) showed that *Cutibacterium* was differentially abundant at the neck site while *Facklamia*, *Eremococcus*, *Finegoldia* and *Corynebacterium* were more abundant at the femoral site.

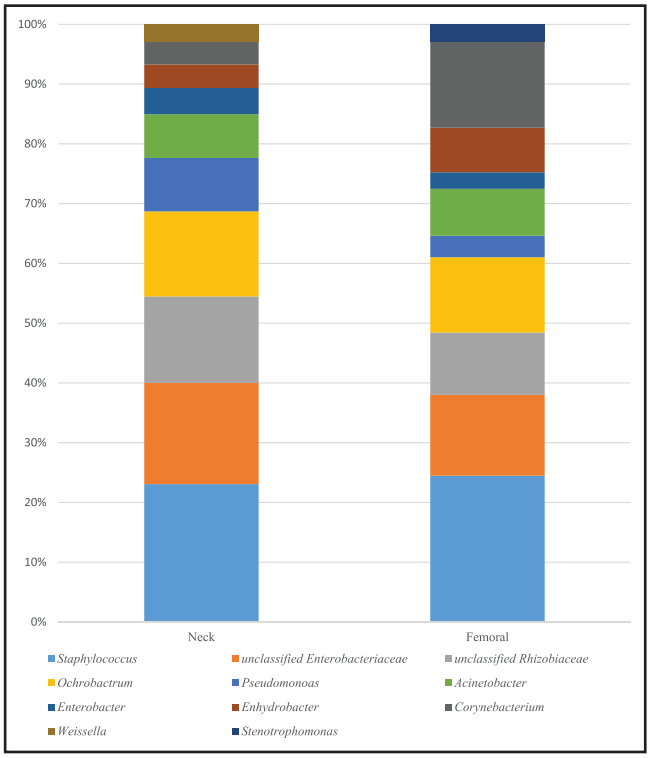

Figure 3. The relative abundance of the top 10 dominant genera from skin swab samples taken at femoral and neck sites of deceased bodies.

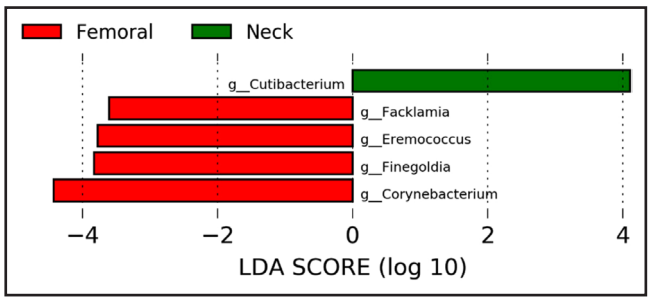

Figure 4. LEfSe showed the differentially abundant genera between neck and femoral site (p-value < 0.05, LDA score ≥ 2.0).

Analysis of relative abundance based on post-mortem interval (PMI)

The duration of the earliest documented time of death to sampling time, known as PMI, was recorded in this study. The PMI of the bodies in this study was grouped into two categories, which were PMI of less than five hours (PMI<5H) and PMI of more than five hours (PMI>5H). Since most of the bodies had the PMI within five hours, five hours was selected as the cut-off. There were 33 bodies that had an PMI of less than five hours, while only seven bodies had an PMI of more than five hours. There should be a total of 66 samples and 14 samples for the PMI<5H and PMI>5H groups, respectively. However, we have excluded three samples due to the sampling depth, which are 1pN and 34pN, two samples from neck site, and 40pF, one sample from femoral site. Thus, there were a total of 64 samples for the PMI<5H group and 13 samples for the PMI>5H group.

Alpha Diversity. The figure showed there is no significant difference between PMI<5H and PMI>5H in Pielou's evenness (Figure 5a, p=0.08), phylogenetic diversity (Figure 5b, p=0.64), observed features (Figure 5c, p=0.47), and Shannon index (Figure 5d, p=0.11) at a sample depth of 5900.

Beta Diversity. PCoA based on weighted UniFrac distance (Figure 6) illustrated clustering of samples by PMI, which were PMI<5H and PMI>5H. The PERMANOVA analysis for weighted UniFrac distance matrix showed that the bacterial composition was detected at both the PMI groups had no significant difference (p-value=0.82).

Relative Abundance. *Staphylococcus* presented as the most dominant genus in both the PMI<5H and the PMI>5H groups (PMI<5H, 22.34%; PMI>5H, 30.41%) (Figure 7). *Ochrobactrum* appeared to be abundant in the PMI<5H group (15.59%); however, it did not rank among the top 10 dominant genera in the PMI>5H group. In contrast, *Corynebacterium* appeared to be the second dominant genus in the PMI>5H group (17.41%). The majority of the genera on the lists were *Proteobacteria*. LEfSe result (Figure 8) showed that *Facklamia* was significantly abundant in PMI>5H group while unclassified *Rhizobiaceae* were more abundant in PMI<5H group. There were significant results shown in LEfSe (Figure 9) while we analysed based on body sites. At the neck site, *Facklamia* and unclassified *Rhizobiaceae* showed the same results as before. However, *Cutibacterium* also showed significant abundance in PMI<5H group at the neck site. On the other side, the genera appeared to be different at the femoral site. In the PMI>5H group, most of the genera belonged to *Firmicutes* except for *Rothia,* which belonged to *Actinobacteriota*.

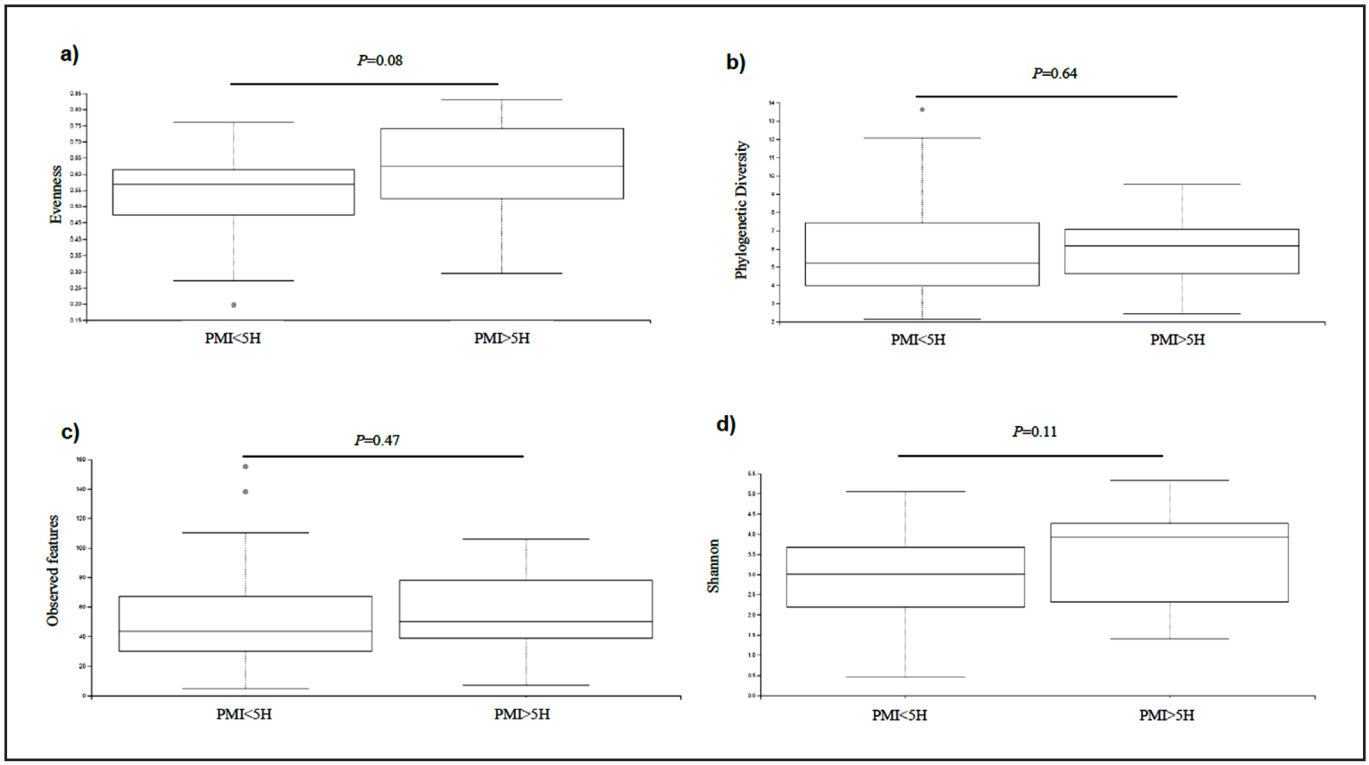

Figure 5. The comparison of alpha diversity indices which are Pielou's evenness (a), phylogenetic diversity (b), observed features (c) and Shannon index (d) between PMI<5H and PMI>5H at sample depth of 5900.

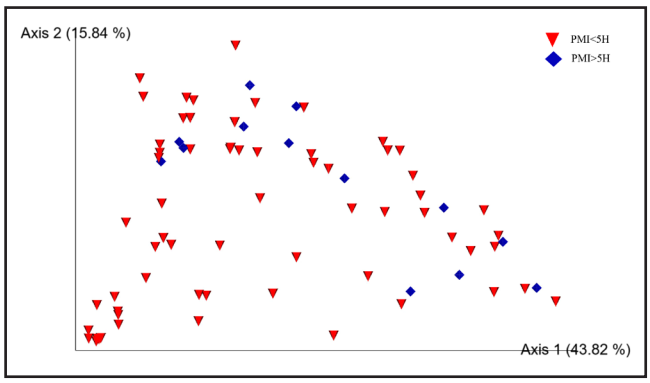

Figure 6. Two-dimensional PCoA plot based on weighted UniFrac distance showed no significant difference between PMI<5H and PMI>5H. Red, PMI<5H (*n*=64); Blue, PMI>5H (*n*=13).

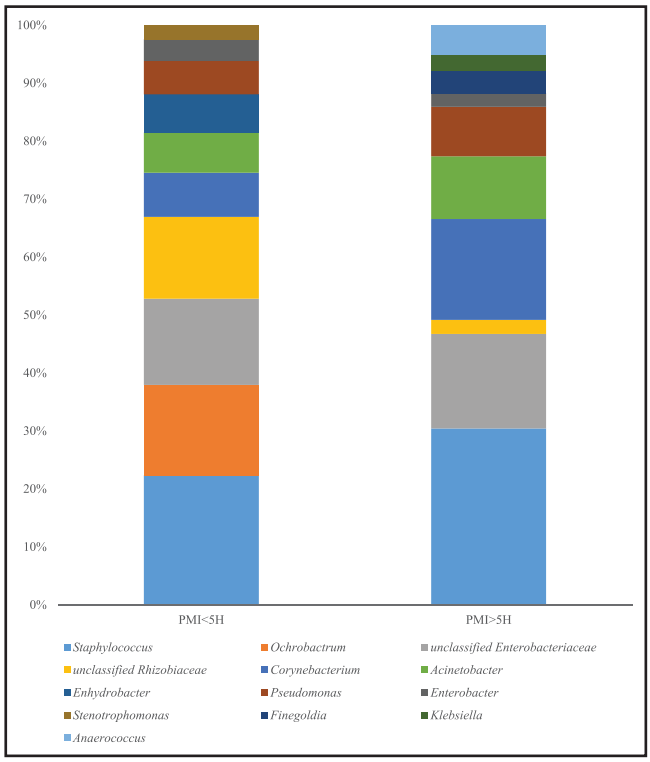

Figure 7. The relative abundance of the top 10 dominant genera identified at PMI<5H and PMI>5H regardless of body sites.

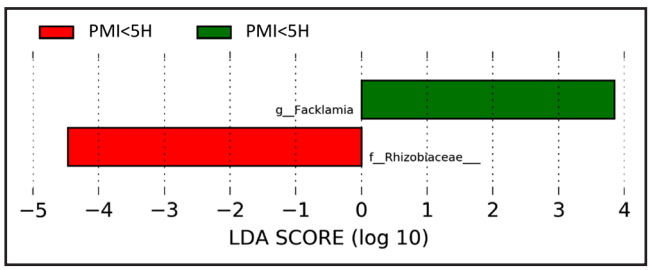

Figure 8. LEfSe showed the differentially abundant genera between PMI<5H and PMI>5H regardless of body sites (p-value < 0.05, LDA score ≥ 2.0).

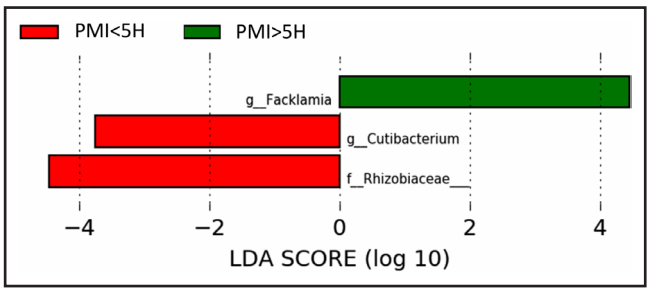

Figure 9a. LEfSe showed the differentially abundant genera between PMI<5H and PMI>5H at neck site (p-value < 0.05, LDA score ≥ 2.0).

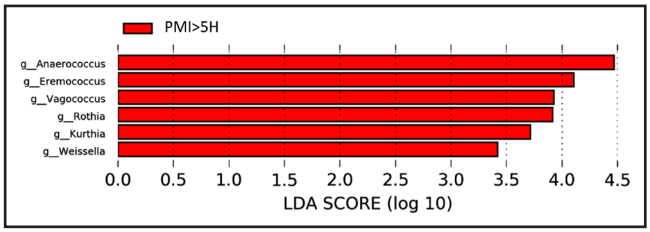

Figure 9b. LEfSe showed the differentially abundant genera between PMI<5H and PMI>5H at femoral site (p-value < 0.05, LDA score ≥ 2.0).

DISCUSSIONS

The phyla *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and/or other taxa are commonly found in normal skin flora, with *Actinobacteria* and *Firmicutes* predominating (Gao *et al*., 2007; Costello *et al*., 2009; Grice *et al*., 2009). Genera such as *Staphylococcus, Corynebacterium*, and *Propionibacterium* are well-known skin commensals that protect human skin (Ogai *et al*., 2018). Bacteria that live on the surface of deceased remains are known as epinecrotic microbiome. In this study, our results were consistent with the findings of the initial epinecrotic microbiome at the beginning of the decomposition process in previous studies. We found that the post-mortem skin microbiome on early deceased bodies at both neck and femoral sites was dominated by *Proteobacteria*, followed by *Firmicutes*, *Actinobacteria*, and a very small abundance of *Bacteroidetes*. Phylum *Proteobacteria* was found on the surface of deceased remains during the initial decomposition process, regardless of the environment in which they decomposed (Metcalf *et al*., 2013; Pechal *et al*., 2013, 2014; Benbow *et al*., 2015; Hyde *et al*., 2015).

Most previous studies performed the decomposition process in natural settings. Factors such as ambient temperature and geographical location are possible to alter bacterial composition during the decomposition process (Grice & Segre, 2011; Blaser *et al*., 2013; Hospodsky *et al*., 2014; Ying *et al*., 2015; Gupta *et al*., 2017). Hyde and her colleagues suggested that even small abiotic effects such as solar irradiance and ambient temperature may influence on the microbial community. The authors reported that the initial phylum that dominated at the skin sites was *Proteobacteria*, followed by *Firmicutes* at the later stage of decomposition (Hyde *et al*., 2015). Although our samples were only within 24 hours of the PMI, we found that genera that belonged to *Firmicutes* were significantly abundant in the PMI>5H group, especially at the femoral site. Moreover, Carter and his colleagues proved that higher temperatures will result in higher microbial activity and a higher rate of decomposition of cadavers (Carter *et al*., 2008). On the

other hand, Malaysia, a country located near the equator, enjoys tropical weather all year long with a hot and stable temperature. The ambient temperature in Malaysia may be a factor affecting the bacterial composition detected on the deceased bodies. Nonetheless, geographical location is also a factor in altering bacterial composition. A previous study found that the predominant bacteria detected in people staying in rural and urban areas of China showed different abundance of *Trabulsiella* and *Propionibacterium* (Ying *et al*., 2015).

The sequencing method may have an effect on the results. *Proteobacteria* was reported to be the dominant phylum detected when using a sequencing-based method. In general, the high throughput sequencing method discovers more Gram-negative bacteria, which mostly originated from the environment, on normal skin. *Proteobacteria* were mostly detected on healthy human skin besides well-known skin commensals, such as *Staphylococcus, Propionibacterium,* and *Corynebacterium* species by using a sequencing-based detection method (Cosseau *et al*., 2016; Procopio *et al*., 2021).

The neck and femoral sites were selected for skin samples as blood samples are commonly obtained from these two sites for PMM examination (Saegeman *et al*., 2009). Our study found that both neck and femoral sites were dominated by the genus *Staphylococcus* then followed by abundance of *Proteobacteria*, which is different from the skin microbiome found on other countries. Human skin is distinguished into different kinds of microenvironments, such as dry, moist, and sebaceous. The neck and femoral sites belong to different kinds of microenvironment. The neck belongs to sebaceous sites while the femoral belongs to moist sites (Grice & Segre, 2011). Different skin areas are dominated by different bacteria (Grice *et al*., 2009; Zeeuwen *et al*., 2012; Ying *et al*., 2015). In the LEfSe results, we found that the genus *Cutibacterium* was significantly abundant at the neck site, while the genus *Corynebacterium* was abundant at the femoral site. Moreover, *Cutibacterium* was found significantly abundant in the PMI<5H group. These findings are similar to those described by Grice and her team in their topographic study (Grice *et al*., 2009), which discovered that *Cutibacterium* and *Staphylococcus* species were more abundant in the sebaceous site, while *Corynebacterium* species dominated the moist site. As the skin microbiome community on the neck is more exposed to the environment, it may be influenced by UV exposure and air pollutants (Patra *et al*., 2016).

PMI, also known as time since death, is one of the main factors that can influence the skin microbiome (Pantopikou & Papasotiriou, 2017; Saegeman *et al*., 2017). Many studies have demonstrated that the bacterial community changes along with PMI (Metcalf *et al*., 2013; Pechal *et al*., 2014; Hyde *et al*., 2015). These authors included the PMI for more than 24 hours in their studies. In this study, bodies within 24 hours of PMI were included and the bodies were separated based on their PMI into two categories, which were PMI<5H and PMI>5H. PMI for 5 h was chosen as the cut-off due to the fact that the samples mainly had the PMI within 5 h. The detected bacterial taxa at the beginning of the decomposition process by previous studies were mainly dominated by the phylum *Proteobacteria*, which was consistent with the finding. Our present study found that class *Gammaproteobacteria* abundant among the samples. Pechal reported that family *Moraxellaceae* significantly abundant on the fresh pig corpses. Our present study also found significant level of *Moraxellaceae* on our samples. In Hyde *et al*. (2015), the authors found that *Acinetobacter* dominated the bacterial community among the skin samples before purge. Our samples were collected from fresh bodies; *Staphylococcus* was found as the most abundant genus in both PMI<5H and PMI>5H groups. However, *Acinetobacter* was included in the top 10 dominant genera among the samples despite the anatomical sites. Furthermore, Pechal *et al*. (2018) stated that the post-mortem microbiome within 48 hours of PMI displayed similar findings as ante-mortem. As all of the samples in this study had the PMI within 24 hours, the skin microbiome detected could be similar as ante-mortem. *Staphylococcus* may be the normal flora that appeared on the skin of deceased bodies.

Instead of Gram-negative bacteria that are mostly associated with the environment, Gram-positive bacteria are often reported as contaminants in PMM examinations (Palmiere *et al*., 2016; Pantopikou & Papasotiriou, 2017). Skin commensals such as coagulase negative staphylococci (CoNS), *Corynebacterium* and *Propionibacterium* species have been identified as contaminants (Miller *et al*., 2018; Fernɒndez-Rodrɒguez, 2019). However, the differentiation between true infection and contamination remains a challenge to forensic pathologists and microbiologists these days. According to previous studies, the authors suggested a few actions to aid in the interpretation of PMM examination results. Two sets of blood cultures are usually obtained to ensure the reliability of the result (Palmiere *et al*., 2016; Fernɒndez-Rodrɒguez, 2019). The time of detection of positive result should be taken into account as contamination mostly happens if the blood culture set shows a positive result after a 1-week incubation (Yu *et al*., 2020). Taking samples from two different body sites can also aid in the interpretation of the results (Christoffersen, 2015). Nevertheless, Gram-positive bacteria can still be opportunistic pathogens. Therefore, the patient's ante-mortem clinical history, forensic pathological examination, and imaging results should be interpreted together to confirm the results (Saegeman *et al*., 2021).

Nevertheless, this study has limitations as it only included a small sample size. More samples can be included for further study, considering more biotic and abiotic factors. Moreover, conventional blood culture can be included for comparison, as high-throughput 16S rRNA sequencing results did not show if the bacteria were alive.

CONCLUSION

With the knowledge of normal skin flora on early deceased bodies, this study is able to aid in the interpretation of PMM results when mixed growth or contamination occurs. However, environmental and personal factors have a strong influence on the bacterial community on deceased remains. More influencing factors and samples should be included in further investigations to ensure reliable and valid findings.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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