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Estimation of Time of Death through Observation of Microbiota Changes in the Oral Cavity

Fahriza R. Oktaviana, Ferry P. Gultom, Roben S. Pasaribu, Elza I. Auerkari

Division of Forensic Odontology, Department of Oral Biology, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia
Email: eauerkari@yahoo.com
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Abstract: Various methods for estimating post-mortem (PMI) have been investigated such as rigor mortis, livor mortis, molecular, chemical, and forensic entomology (insect) methods. However, these methods have weaknesses, therefore researchers are looking for more accurate methods in determining the time of death of a person. Molecular methods are unreliable due to the degradation of DNA, RNA, and proteins in corpses over time. In different parts of the body, the amount of diversity of microbes will be different. The oral cavity is the most abundant microbial area among other parts of the body due to its continuous exposure to the respiratory and digestive systems. This area has great potential in estimating time intervals of death because of its ease of access and the types of microbiomes that predominate at any given time. Therefore, this narrative review was conducted to describe studies that used oral microbiota communities to estimate post-mortem intervals. *Proteobacteria, Firmicutes, Actinobacteria*, and *Bacteroidetes* are the dominant microbial types found in corpses. *Firmicutes* became one of the dominant bacterial phyla in the early stages of decomposition. *Actinobacteria* were found to decrease as PMI increased. Studies have shown that the oral microbiome has excellent potential as a parameter to determine the post-mortem interval. However, further research is needed with more complex environmental conditions such as different humidity and temperature. In addition, further research requires more samples of human remains to obtain more valid results.
Keywords: oral microbiome; post-mortem interval; human identification; forensic odontology

Introduction

Death is the end of a physiological process in which cells are no longer able to maintain their function and integrity, followed by changes both chemically and physically.[1] In forensics, determining the length of time between death and the time when the corpse is found (post-mortem interval) will assist in making death certificates, writing wills, and death schemes for a corpse. Estimation of PMI by observing biological changes that occur in corpses such as insect activity (forensic entomology), rigor mortis, livor mortis, molecular, and chemical are methods that are often applied to determine the death of a human being in days, weeks, or months. Molecular methods are unreliable due to the degradation of DNA, RNA, and proteins in corpses over time. Another method is by looking at the last communication made before he died, one of which is via a cell phone. However, these methods are too subjective, limited in time accuracy, and sensitive because they depend on the environmental conditions around the corpse, and the estimated time of death is too broad.[2–5]

Forensic microbiologists seek to develop methods of determining death by observing the changes in the microbiota that occur when a person dies. The microbiota community in the decomposition process will experience changes as cell autolysis and chemical degradation occur so that it can be used to estimate PMI. Two kinds of post-mortem microbiota communities can be observed in corpses when differentiated from their location, namely the thanatomicrobome and epinecrotic communities. The thanatomicrobiome studies changes in the community of microorganisms in internal organs (heart, liver, intestines, brain, spleen, and others) after death, while the epinecrotic microbial community studies prokaryotes, fungi, protists and microeukaryotes that grow on the surface of corpses (superficial epithelial tissue, oral mucous membranes, and the distal orifice of the digestive tract).[5,6] Numerous studies have investigated microbes for the estimation of time to death with a wide variety of samples, ranging from dead animals to human cadavers.[5,7]

The oral cavity consists of teeth, keratinized (hard palate and gingiva) and nonkeratinized tissues (lips, soft palate, cheeks), tongue, tonsils, and gingival sulcus which act as a link to the digestive and respiratory tracts. These parts become a place for various microorganisms such as bacteria, fungi, and viruses to colonize which are then called the oral microbiome.[8] This community is very important for humans because it functions as a normal flora in the oral cavity and if there is an imbalance it will cause dental caries disease, inflammation of the gingival and periodontal, as well as oral and systemic (gastrointestinal and nervous) mucosal diseases.[9] The oral microbial community is the second most microbial area after the colon, with about 100 bacterial species identified. Some of them are from the phyla *Proteobacteria, Actinomycetes, Spirochetes, Firmicutes, Bacteroidetes,* and *Aponeurophytes*. Both oral and gastrointestinal community bacteria have significant roles in decomposition.[1] However, the oral microbiome has received less attention than the gut microbiome because there has been little research related to this community.[10] Based on this, this narrative review was undertaken to describe the use of the oral microbiota community to estimate post-mortem intervals.

Oral Microbiome

The human oral cavity is one of the habitats of a complex microbial community in which the second most abundant microbiome contains microorganisms after the gut microbiome (intestine). This is because the oral cavity is continuously exposed to microbes from the respiratory and digestive systems. The oral microbiome maintains the balance and health of the oral cavity. If this balance is disturbed, periodontal disease, endodontics caused by caries, tonsillitis, and osteitis will appear. In addition, systemic diseases such as cardiovascular disease, diabetes, pneumonia, premature birth, obesity, colon carcinoma, and psychological disorders are also correlated with changes in the balance of the oral microbiome.[11]

In the oral cavity, there are about 1000 species of microorganisms such as bacteria, viruses, fungi, protozoa, and archaea.[11] Several viruses are associated with diseases, namely mumps,

rabies, hepatitis, HIV, and HPV where each has clinical manifestations and different locations, in the oral cavity. Protozoa species such as *Entamoeba gingivalis* and *Trichomonas tenax* are normal amoeba found in the oral cavity. Poor oral health and the presence of gingival disease can increase the number of these microorganisms. Fungus is also a normal flora, but it can cause both acute and chronic infections. The most dominant fungal genera in the oral cavity are *Candida, Aspergillus, Saccharomycetales, Aureobasidium, Fusarium, Cladosporium,* and *Cryptococcus*. Minor components such as archaea can be detected by a small number of species in the oral microbiome such as *Methanosarcina mazeii* and *Methanobacterium curvum/congolense, Methanibrevibacter oralis* and other unidentified *Methanobrevibacter phylotypes*. Their number and prevalence increase in subjects with periodontitis.[12]

The most dominant bacterial communities in the oral microbiome are the phyla *Bacteroidetes, Firmicutes, Actinobacteria, Spirochaetes, Fusobacteria,* and *Proteobacteria* where these bacteria are included in 96% of the detected species.[12] The order of the most abundant bacteria in the oral cavity is, as follows:[13,14] *Firmicutes* (36.7%), *Bacteroidetes* (17.1%), *Proteobacteria* (17.1%), *Actinobacteria* (11.6%), *Spirochaetes* (7.9%); and *Fusobacteria* (5.2%).

Phylum such as *Chlamydia, Chloroflexi, Tenericutes, Euryarchaeota, Synergistetes,* SR1, TM7 are small, namely around 4% of the total bacteria in the oral cavity. *Firmicutes* dominate the oral cavity of healthy people, while the *Bacilli* from *Firmicutes* that are mostly found are in the *Streptococci* genus (19.2%) followed by *Veilonella* (8.6%). *Prevotella* are most numerous on the lateral and dorsal surfaces of the tongue. *Porphyromonas gingivalis* and *Captocytophaga* are examples of *Bacteroidetes* that live in healthy oral microbiota. *α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, δ-Proteobacteria, ε-Proteobacteria* are five classes of *Proteobacteria* detected in the mouth. However, only *γ-Proteobacteria* are contained in saliva based on NGS analysis. This phylum is more numerous if a person has a disease of the oral cavity. Examples of genera of Actinobacteria that live in healthy people are *Actinomyces, Atopobium, Corynebacterium* and *Rothia. Spirochaetes* belong to a phylum of the oral microbiome that are rarely cultured. As many as 70% of the species have not been cultured. *Spirillaceae* and *Leptospiraceae* are the most common *Spirochaetes* found in healthy human oral cavity. *Fusobacteria* such as *Fusobacteriaceae* and *Leptotrichiaceae* are included in late colonizers in the mouths of healthy people.[14]

Human Post-mortem Microbiomes

Post-mortem interval (PMI) is the determination of the time of death when there are no other witnesses. Various methods for determining the time of death have been developed, such as by looking at the last communication (cell phone, letters, visual sighting, etc.) and biological conditions (rigor mortis, livor mortis, insect activity, etc.). These methods are very limited in application because they can only estimate the time in days, weeks, or months. Most studies of the human microbiome examine microorganisms related to human health (commensal and pathogenic) but not too many in the post-mortem state. The study of microorganisms in the condition of dead humans has the potential to develop the field of forensic microbiology because of its use to estimate the time of death of a person. This study shows that in human/animal deaths, antemortem bacteria can still be found in the corpse.[5,6]

The decomposition process of corpses is influenced by many factors, for example biotic factors (extrinsic and intrinsic bacteria and other microbes, insects, and special characteristics that only cadaver have), abiotic factors (humidity, climate, and weather), and ecological conditions. In the initial decay process, the intrinsic bacteria digest the gut from within and then start digesting the surrounding tissue. Reduced oxygen levels in the corpse make dead cells undergo autolysis where enzymes begin to break down. In addition, there is also a process of changing aerobic bacteria to become anaerobic (anaerobic respiration) and releasing gases as by-products such as methane, puterscine, hydrogen sulfide, and cadaverine. The buildup of these gases compresses and expands the cadaver and then forces the liquid out.[5,15,16]

There are two types of post-mortem microbiome in humans, namely the thanatomicrobiome

and the epinecrotic microbiome. The microorganisms studied in the thanatomicrobiome are internal organs such as the brain, spleen, heart, intestines, liver, and others.[5,6] Among these organs, the highest microbial diversity lies in the liver according to Can et al.[17] Another community included in the post-mortem microbiome of humans is the epinecrotic microbial community. Microorganisms in this community are protists, prokaryotes, fungi, and other microeukaryotes that live on the surface of the corpse. Superficial epithelial tissue, oral mucous membranes, and the distal orifices of the digestive tract are the surfaces with which the microbial community lives. Several studies took samples with swabs on these surfaces and the results showed that there was high microbial biodiversity and it differed between individuals. Research on post-mortem microorganisms typically uses animal models such as pigs and rats or human cadavers that are donated or have been autopsied in criminal cases. However, cadaveric use is difficult to replicate when compared to animal studies.[5,6]

Several studies have examined microbes for the estimation of time to death with a wide variety of samples, from dead animals to human cadavers. Metcalf et al[7] examined thanatomicrobiomes and epinecrotic community in the abdominal cavity and skin of rats that had died and decayed, and soil used for burial for 3-48 days. At the bloat stage, anaerobic bacteria such as *Lactobacillaceae* and *Bacteroidaceae* increased, followed by the dominance of aerobic and facultative anaerobic bacteria such as *Enterobacteriaceae* after rupture. An epinecrotic study on pig skin conducted by Pechal et al., 2014 found that at the beginning of the decomposition stage bacteria were the most dominant. phylum *Proteobacteria*. Meanwhile, in the final stage, the most numerous phyla are *Firmicutes*.[18]

Methods of Microbial Community Analysis at the Genom Level

Profiling of the microbial community is limited because most bacteria are difficult to isolate or culture in the laboratory. Various methods have been used to study bacterial communities for decades such as Denaturing Gradient Gel Electrophoresis (DGGE), Terminal Restriction Fragment Length Polymorphism (T-RFLP), Fluorescent in Situ Hybridization (FISH), and Genechips. However, now high-throughput sequencing technologies, namely Roche 454 and Illumina, are more widely used because they can identify bacteria that cannot be cultured.[19]

Two methods are often used to analyze microbial communities at the genomic level, namely gene surveys with 16S rRNA and metagenomics bioinformatics. The 16S rRNA survey method is considered affordable with good laboratory procedures and bioinformatic design. However, this method only provides a composition of low taxonomic resolution without functional information. On the other hand, metagenomics bioinformatics can provide functional information on microorganisms at the strain level but the analysis is very complex and the available databases regarding human microbial species are incomplete. Three techniques are often used in metagenome sequencing or segments of the metagenome, namely 16S ribosomal RNA sequencing, shotgun sequencing, and pyrosequencing.[20]

The amplicon sequence of the 16S rRNA gene is a reliable biomarker that is often used for phylogenetic analysis and taxonomic classification of the post-mortem microbiome in forensic microbiology.[21] The 16S rRNA sequence was first introduced in 1987 by Carl Woese to identify bacteria and elucidate bacterial domain relationships. V_1, V_2, V_3, and V_4 are the most diverse regions and are often used in studying bacterial diversity among the nine hypervariable regions because they can describe the entire bacterial phylum present in a niche. V_1 region is widely used to differentiate *Streptococcus* colonization, V_2 to identify *Fusobacterium* and *Porphyromonas* (Gram-negative), while V_5 has less diversity than V_{1-5}. Region V_{1-3} at the time of amplification showed the presence of *Prevotella, Streptococcus, Porphyromonas, Treponema, and Bacteroides* while V_{4-6} showed the dominance of *Treponema, Campilobacter, Prevotella, Porphyromonas*, and *Enterococci* bacteria. The V_{4-6} region is considered the most widely used to study the entire bacterial phylum along the 16S rRNA because it is very reliable, except for detecting *Fusobacterium.*[14] According to Yang et al[19] 2016, the V_{4-6} combination which is classified as

Class I can represent this subregion as a phylogenetic bacteria study from the new phyla because it has the highest sensitivity compared to Class II (V_3 and V_7) and Class III (V_2 and V_8). In addition, Class I is the main functional part of the 16S rRNA because it includes the "690 hairpin" and the coding center. "690 hairpins" are found in three phylogenetic domains whose loops are very well preserved, namely in the V_4 16S rRNA region. The procedure includes amplification and cloning sequencing with PCR to view DNA segments. The purpose of DNA sequencing in this method is to identify species. 16S rRNA sequencing is considered fast, accurate and can detect viral DNA, however, PCR is very sensitive to contamination and prone to bias.[22]

The Home Oral Microbiome Database (HOMD) is the first database to describe and provide insight into the oral microbiome and its importance in human health. HOMD is a 16S rRNA gene-based data that provides scientific information about 700 species of prokaryotes that have been in the human oral cavity for the last two decades. This database contains more than 35,000 clone sequences and 600 gene libraries of 16S rRNA. HOMD links sequence data with phenotypic, clinical, phylogenetic, and bibliographic information. Of the 700 species identified by HOMD, only 51% have been named, 13% have not been named but have been cultured and 28% have not been named and cultured. In addition, HOMD contains 150 genera, genomes for 400 oral taxa, and about 1300 strains of microorganisms. HOMD also contains genomes for 202 strains and 30 *Streptococcus* taxa. The weakness of HOMD is that the data in it show that 20-60% of microorganisms cannot be cultured even though this database is based on microorganism culture.[9]

The metagenomics bioinformatics method can characterize the entire microbial community both from the composition at the strain level and functional profiling of identified and unidentified species.[20] Extraction of the total DNA samples obtained by this method is then performed by shotgun sequencing without carrying out the PCR amplification step. The shotgun sequencing procedure is by fragmenting and sequencing long DNA randomly several times to read overlap from the target DNA using a computer program. [22] This method is rarely used because it is more expensive than the amplicon-sequencing survey method and more difficult to do, especially when the environment being studied contains many DNA of the host as in the mucosa-associated microbiome. This method is very complex because it requires knowledge in terms of programming and bioinformatics as well as high computer technology to analyse data.[20] The advantage of shotgun sequencing is that it can see metabolic activity and also does not require much human intervention. Pyrosequencing is very good at determining biodiversity, produces many sequences and is unbiased because it doesn't need cloning. However, this method is the most expensive among the other options and does not produce the full 16S rRNA sequence required for taxonomic studies.[22]

Oral Microbiome in Estimating the Post-mortem Interval

Various studies related to microbiome analysis on parts of the human body have been carried out to measure the post-mortem interval more accurately.[2] The following shows the percentages of bacterial cells in human body from the highest to the lowest, namely the digestive tract (29%), oral cavity (26%), skin (21%), respiratory tract (14%), urogenital tract (9%), and blood (1%).[23] The oral cavity is widely used as an object of research because of its easy access without surgery. Several studies have shown that the oral microbial community can be distinguished from the early and late stages of decomposition, so that, it has the potential to be used as a benchmark in determining the post-mortem interval.[2]

The microbial community of an individual is very influential both from intrinsic and extrinsic factors such as age, sex, lifestyle, eating patterns, clothes worn at death, physiology, health status and the environment around the corpse. There are various methods of identification of bacteria. Culture methods can identify some bacterial species but only a small part of the human microbiome. Sequencing methods such as 16S rRNA, metagenomic and meta-transcriptomic sequencing are the latest methods in analyzing the diversity of the microbiome in PMI when decomposition occurs.[2,23]

Several microbiological studies related to post-mortem use experimental animals or human

cadavers as research models. Direct post-mortem microbiological assessment of cadavers is better, but it is difficult to control PMI and the number of replications is very limited. However, this weakness can be overcome by using experimental animals. Rats and pigs are the most widely used animals in epinecrotic microbial community research because their decomposition process is like humans.[6] In this review, several studies regarding the use of oral microbiomes are summarized to estimate post-mortem intervals which are then divided into two research groups, namely experimental animals. and cadaver.

In 2019, Dong et al[1] collected 24 rat oral samples for 240 hours post death. Samples were taken from three female rats and three male rats which were sacrificed in the time groups 0 hours, 24 hours, 144 hours, and 240 hours after death. Significant changes often occur within 24 hours after death, which is the halfway point between the early and late PMI phases. There was only one sample that failed to be sequenced, namely one male rat, while the other 23 samples were successful. Samples with a large number can minimize errors during research and assess how much intra-individual microbiota variation in humans and other mammals. However, in a study conducted by Yang et al[19] with an animal model validity test on skeletal muscle buried with soil showed that no network was the best at being a predictor of representing human tissue.

Another study conducted by Zhao et al[15] also used rats as models in the study, namely 96 male rats. The bodies of the rats that had been sacrificed were then placed in a room with a temperature of 23-26° C to resemble the decomposition process. Samples of the oral swab were taken at the following times: 0 hours, day 1, day 3, day 5, day 10, day 15, day 20, day 24, day 30, day 40, day 52 and day 59. Each hour consisted of 12 rats. In this study, the swab areas were the tongue, palate, tooth surface and buccal (cheek) mucosa. One of the reasons for choosing this time was because of the stiffness of the corpse which disappeared after 16 hours and on the third day the gas accumulated in the abdomen and body causing rupture and decomposition fluids to flow out.

Direct observation of the oral microbiome in cadaver was carried out by Adserias-Garriga et al[23] in 2017. In that study only three donated cadavers were used due to research time limitations. All data including age at death, sex, dental and general condition were taken from each cadaver. Then data on temperature, vegetation, the presence or absence of insects and weather conditions were also recorded. The first cadaver was an 80-year-old white male with a fixed crown on the anterior of the upper jaw, the second 81-year-old female, no teeth, and the third 27-year-old female without dentures. Cadavers at each stage of decomposition (fresh, bloat, active decay, advanced decay and putrid dry remains) were documented. All three cadavers showed the same features at each stage. At 21-27° C and 71-95% humidity, oral swab samples were taken from each stage of decomposition, namely on the tooth surface (occlusal, lingual, and buccal), tongue, palate and buccal mucosa. The double swab technique was used in this study.

Ashe et al[2] also studied a cadaveric model, namely one male and two females. The first cadaver (male) wore dentures, the second used complete dentures with metal components, and the third cadaver had no teeth at all. All cadavers were treated the same, namely they were not wearing clothes, placed supine on the ground without anything. In contrast to previous studies, the cadavers were placed outdoors facing the humid environmental conditions of the area. Oral swabs were performed over the entire hard palate for two months.

Table 1 summarizes the studies studying the oral microbiome as a means of estimating the post-mortem interval.

Table 1. Research related to oral microbiome to estimate post-mortem interval

Authors, year	Research Design
Adserias-Garriga et al, 2017[23]	Research samples: three donors Donor 1: 80-year-old white male with crown on upper anterior teeth Donor 2: 81-year-old edentulous white female Donor 3: 27-year-old white female with no history of dental work The cause of death of all donors was not due to infection and nothing was preserved.

	Research methods: Double swabbing of palate, tongue, buccal mucosa, teeth surfaces (buccal, lingual, and occlusal surfaces) Bioinformatic analysis: 16S rRNA sequencing Results: 1. Fresh stage: *Firmicutes* and *Actinobacteria* dominant but decreasing over time (Day 1–5–6), dominated by *Lactobacillaceae, Staphylococcaceae, Gemellaceae, Carno-bacteriaceae, Aerococcaceae, Veillonellaceae, Streptococcaceae, Campylobactera-ceae, Micrococcaceae, Bifidobacteriaceae, Actinomycetaceae* and *Coryne-bacteriaceae* 2. Bloat stage: *Tenericutes* (Day 5-7); *Peptostreptococcaceae, Bacteroidaceae, Enterococcaceae; Clostridiales* (mulai muncul di beberapa hari terakhir) 3. Advanced decay: *Firmicutes (Clostridiales* and *Bacillaceae*) (Day 6-12); *Ignatzschineria* (*Gammaproteobacteria*), *Pseudomonadaceae, Alcaligenaceae, Planococcaceae* increased. 4. Putrid dry remains: *Bacilli* and *Clostridia* (Day-12) Conclusion: this research is a preliminary study regarding the use microorganism to estimate time of death.
Dong et al, 2019[1]	Research samples: 24 mice Research methods: Cotton swabs that have been sterilized at high temperature and moistened with aseptic water are then rubbed into the oral cavity of the rats that have been sacrificed with carbon dioxide gas. Bioinformatic analysis: 16S rRNA sequencing Results: • 0 hours: dominated by phyla *Proteobacteria, Firmicutes, Actinobacteria* and *Bacteroidetes* • 24 h (early PMI): *Firmicutes* increases first 24 hours then decreases, predominated by *Bacilli, Lactobacillales, Streptococcaceae* and *Streptococcus* • More than 24 h (late PMI): *Enterobactericeae* (*Serratia, Escherichia, Klebsiella* and *Proteus*) • Predominates the post-mortem interval: *Gamma-proteobacteria* Conclusion: *Gamma-proteobacteria* and *Proteus* have a great potential in the oral microbial community for PMI estimation
Ashe et al, 2021[2]	Research samples: three donors Donor 1: male, wearing dentures. Donor 2: female, wearing complete denture with metal components Donor 3: female, edentulous Research methods: All donors were situated on the ground in a supine position and each donor swabbed the entire surface of the hard palate. Swabs were carried out 5-7 times according to the decomposition process. Bioinformatic analysis: 16S rRNA sequencing, whole metagenomic (MetaG) and metatranscriptomic (MetaT) sequencing, culture Results: • Dominant: *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* but the distribution is not good enough to distinguish stages or times • Key genera observed at the genus level: *Rothia*; *Firmicutes* (*Lysinibacillus*, *Lactobacillus*, *Staphylococcus*); fungi (*Candida* and *Yarrowia*) • Bacteria found in all methods: *Acinetobacter gerneri*, *Proteus vulgaris*, *Morganella morganii*, *Pseudomonas koreensis*, *Pseudomonas moraviensis*, *Comamonas terrigena*, *Raoutella terrigena*, *Stenotrophomonas maltophilia*, *Bacillus cereus*, *Lactobacillus paracasei*, and *Kurthia zopfii* Conclusion: there is no relationship between PMI length and bacterial community at a given time possibly due to influencing factors such as humidity, temperature, sample size, decay insect activity, sex, body weight and cause of death.
Zhao et al, 2022[15]	Research samples: 96 rats were divided into groups of 0 hours, 1st day, 3rd, 5th, 10th, 15th, 20th, 24th, 30th, 40th, 52nd, 59th. Each group consisted of eight rats. Research methods: Swab on palate, tongue, buccal mucosa, and tooth surfaces Bioinformatic analysis: 16S rRNA sequencing Results: • Bacteria found: *Lactobacilles, Enterobacteriaceae*, *Bacteroidetes*, *P. mirabilis*, *Gammaproteobacteria*, *I. indica*, *Vagococcus lutrea*, *Rhodanobacter Lindaniclasticus*, *Bacillales*, and *Cerabacillus*

	• Dominant phyla: *Proteobacteria, Bacteroidetes, Firmicutes,* and *Actinobacteria* • Important genera: *Cerasibacillus, Anaerosalibacter, Proteus, Ignatzschineria* • Important species: *A. bizertensis, P. mirabilis, V. lutrae, B. fragilis, I. indica* and *E. faecalis* Day 1: dominated by phyla *Proteobacteria, Firmicutes* and *Actinobacteria* (peaking 1 hour after death); genus *Enterococcus* followed by *Acinetobacter; Staphylococcus* increased. Day 5: *Bacteroides* Day 5, 10 and 15: dominated by *Ignatzschineria (Gammaproteobacteria)* Conclusion: the structure of the microbial community changed during the cadaveric decomposition process, starting with a decrease in the number of phyla, genera and species then increasing where the change occurred on the 24th day

Firmicutes is one of the bacterial phyla that predominate in the early stages of decay. In the advanced decomposition stage, this phylum is separated from its cluster with *Actinobacteria*. This is because *Firmicutes* are opportunistic bacteria that degrade organic matter from the availability of oxygen. After oxygen is used up and decay enters the bloat stage, there is a change in composition from aerobic bacteria to anaerobic bacteria.[1,2,15,23] Examples of the *Firmicutes* phylum are *Streptococcus, Staphylococcus, Lysinibacillus, Lactobacillus*, and *Vagococcus*.[2] However, in a study conducted by Dong et al,[1] it was stated that the *Firmicutes* phylum did not have a significant linear relationship with the post-mortem interval.

Proteobacteria in the bacterial kingdom is the broadest phylum of the Gram-negative type. In all studies regarding oral microbiomes as a post-mortem interval, the *Proteobacteria* phylum is the most dominant, but it should be noted that the relationship between microbes and PMI is not the same between families and orders of the *Proteobacteria* phylum. The bacteria *Proteus, Proteobacteria, Gamma-proteobacteria, Enterobacteriaceae, Enterobacteriales,* and *Oblitimonas* are examples of bacteria in this phylum.[1,2,15,23] *Proteus* is a facultative anaerobe because it is related to the closed mouth condition of rats when they die. After the dead body ruptures, *Enterobacteriaceae* bacteria (*Klebsiella, Proteus, Serratia, Escherichia*) become more abundant. According to Dong et al,[1] *Enterobacteriaceae* is one of the bacteria in the digestive system that also dominates the oral microbiome at the late PMI stage. *Oblitimonas* in Ashe et al 2021's[2] study appears at the advanced decomposition stage. Aerobic metabolism plays an important role in diversity and bacterial colonization at the fresh stage of decomposition. The process of consuming oxygen by aerobic metabolism is carried out by fermentative bacteria such as *Proteobacteria* and *Tenericutes*.[23] *Proteus mirabilis* is one of the bacteria whose number increases on the fifth day of decay by producing urease which hydrolyzes urea and increases the pH. Therefore, the smell of urea is wafted at that stage. *Gamma-proteobacteria* (*Ignatzschineria*) is a type of *proteobacteria* that predominates on days 5, 10 and 15 where time has the potential to be an indicator of specific post-mortem time.[15] Dong et al[1] stated in their research that *Proteus* and *Gamma-proteobacteria* had great potential as bacteria to predict the PMI of the oral microbial community especially when combined with *Enterobactericeae* in the digestive system.

In a study by Ashe et al,[2] *Actinobacteria* was one of the most common bacteria found when tested with three methods (16S rRNA, MetaT and MetaG sequencing). In the 16S rRNA sequencing method, this bacterium has the highest score.[2] However, the *Actinobacteria* phylum was found to decrease with increasing PMI. In the early stages of decomposition, *Actinobacteria* predominated, then began to form separate clusters in the advanced decomposition stage.[23] *Actinobacteria* live in aquatic and terrestrial ecosystems, especially soil, and play a role in recycling heat-resistant materials through the formation of humus and decomposition.[1] According to Adserias-Garaga et al,[23] bacteria of the *Tenericutes* phylum appeared at the bloat stage, which was on the fifth to seventh day due to changes in nutritional and physico-chemical conditions to match the environment. *Tenericutes* are parasitic/commensal bacteria in their hosts (eukaryotic) without cell walls that live in the gastro-intestinal tract and can be in the oral cavity during the bloat stage. At this stage these bacteria always appear because when the host lacks the tricarboxylic acid cycle, these bacteria use anaerobic fermentation of simple sugars for substrate-

level phosphorylation. Research from Ashe et al[2] mentioned the genera that grew with increasing time of death, namely *Ignatzschineria, Lysinibacillus, Vagococcus,* and *Yarrowia.*

Conclusion

Along with the advancement of technology, microgenomics has been more accurate in detecting microbes, resulting in this method being more desirable to researchers than other methods. Most of the studies used the double swab technique to obtain the microbes from the samples. Various studies related to the determination of the post-mortem interval of the oral microbiome have shown different results. However, differences can be obtained from a comparison between the early and late stages of decomposition. Research with cadavers is better than experimental animals, but limitations in obtaining corpses according to the criteria and time of study are still a weakness in research related to post-mortem intervals. In addition, studies are needed in various conditions such as differences in temperature, humidity, and conditions of corpses for further research.

Conflict of Interest

The authors declare no competing interests.

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