Analysis of Changes in Oral Microbiota for Interval of Post-Mortem Estimation: Review Article

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ABSTRACT

Background: *The microbiota change caused by the decomposition process after death can be used to determine the post-mortem interval. This condition is associated with changes in the oral microbiota. Oral microbiota can be used as an appropriate source for analysis without interfering with the corpse or breaking Indonesian law. This review will examine the changes in the oral microbiota to determine the time of death.*
Case: *The literature search was carried out using PubMed, ScienceDirect, and Google Scholar, and the results were screened based on the inclusion and exclusion criteria**.** The discussion was based on six literature reviews that met the criteria and subject matter of this review.*
Conclusion*: The microbiota in the body contributes to the decomposition process after death. This condition allows it to be used as a reference for determining the postmortem interval. These changes, however, cannot be distinguished from the presence of factors such as temperature, pH, humidity, and an anaerobic environment. Consider these factors when analyzing post-mortem intervals using oral microbiota changes.*

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INTRODUCTION

Forensic microbiology is a subfield of forensic science that is still evolving. Forensic microbiology employs molecular biology, biochemical analysis, and genetic analysis in legal proceedings. Microbiota are used as physical evidence in forensic cases because they can be found anywhere and are specific to certain environments. Microbiota analysis can be proven and used as physical evidence in legal cases using specific tools. [1] Forensic microbiology gained popularity after it was used in the 2001 anthrax bioterrorism attack in the United States. [2] Microorganisms have the ability to respond quickly to changes in their environment. As a result of this situation, microorganisms are used as physical evidence in forensic cases such as supporting crime scene examinations, detecting microorganism-caused deaths, locating trace evidence, and estimating post-mortem intervals. [3,4] The microbiota changes as a result of this process. decomposition, where physico-chemical changes will occur due to the role of microbiota in the body. Temperature and humidity will also affect the decomposition process and the microbiota that live within it. [4]

Decomposition is a natural process that occurs after death, beginning with cellular changes and progressing macroscopically until post-mortem changes appear. [5] In general, decomposition is divided into two stages: autolysis and putrefaction. The autolysis stage occurs when hydrolytic enzymes derived from lysed body cells cause tissue breakdown. The degradation process continues at the putrfaction stage, with the involvement of microorganisms such as bacteria, fungi, and protozoa. [5,6] Changes that occur after death are influenced by a variety of environmental factors, which will affect changes in the microbiota ecosystem during the decomposition process. Temperature, pH, nutrients, and oxygen levels are all important considerations. Several studies have been conducted to examine the relationship between changes in the oral microbiota and the estimated post-mortem interval. [7-11] According to these studies, several bacteria, including *Proteus mirabilis, Ignatzschineria larvae, Pseudomonas fragii, Clostridium estherticum*, and others, can be used to estimate the time of death. Other gamma-proteobacteria. Methods for estimating postmortem intervals are still being developed due to the numerous factors that can influence the decomposition process. [11] Other bacteria, such as Streptococcus sanguinis and Veilonella atypica, predominate in the oral cavity ecosystem, whereas it is known that the first 24 hours experienced an increase. Meanwhile, the *Proteus mirabilis* bacteria will increase after 24 hours post-mortem, and the number will continue to rise with the time interval of death.[12]

The post-mortem interval is an important aspect in forensic analysis because it is related to law, such as in medicolegal cases, and is used to provide an overview of the suspect's or victim's alibi, as well as for death certificates and inheritance rights distribution. [7] In general, there are several methods for estimating the time of death, including measuring the body's temperature, using entomological methods, and visually analyzing post-mortem changes such as algor mortis, livor mortis, rigor mortis, and tissue discoloration. [6] Understanding post-mortem interval changes is critical for accurately estimating PMI and determining the time of death. This situation necessitates taking into account factors that will influence the decomposition process, so several studies have conducted additional molecular analyses of PMI determination, specifically looking at changes that

occur in RNA [13], DNA [14], and protein [15,16]. Other research has also been conducted. in various subject conditions, such as buried corpses [17,18], test animal carcasses in the summer [9], and winter [11]. Based on the foregoing, PMI estimates are still in development, and additional research is required, particularly in Indonesia. Because Indonesia has a tropical climate, a number of factors are expected to differ from previous international studies. There has never been research on changes in the oral microbiota after death to analyze PMI estimates in Indonesia, which is related to the Indonesian law on the use of corpses. In this review, we will look at the changes in the oral microbiota and how they relate to the post-mortem interval.

LITERATURE REVIEW

The literature review was limited to the years 2015–2023, and the language used was English. This review will include all research, case reports, cohort, and cross-sectional studies on microbiology forensics in the decomposition process of the human body or animal laboratory. The authors used four databases, including PubMed, ScienceDirect, and MEDLINE. Several studies have been conducted to investigate changes in oral microbiology with time of death. Microbiological methods have been used to estimate post-mortem intervals in pig and mouse samples, as well as in human corpses donated for research purposes. Researchers examined microbiological changes at post-mortem intervals in several areas, including the oral cavity and gastrointestinal tract, which have the highest microbiota in the human body. Table 1 provides a summary of research on oral microbiological changes and estimated post-mortem intervals.

Author	Samples	Interval of Post-Mortem	Result
Dong et al (2019)	Intraoral swab of 24 adult mice	At death time, 24 hours and 240 hours after death	The results of data on changes in oral microbiology are differentiated based on the taxonomic classification of bacteria. The data results that have relative values to the post-mortem interval based on bacterial phylum are Proteobacteria, while based on bacterial class, Gamma-proteobacteria are obtained. At the bacterial order level we get Enterobacteriales, while at the family level we get Enterobacteriaceae. At the genera level, Proteus bacteria were found to have values relative to the post-mortem interval. [7]
Garriga et al (2017)	Intraoral swab of 3 cadavers (1 man and 2 females)	Payne classification	From the beginning of the research, the results showed that bacteria were dominated by the Actinobacteria and Firmicutes phyla, which then at the initial damage stage was dominated by the Firmicutes phylum. [8]
Guo et al (2016)	Swab of mucosa buccal in 18 Adult Sprague Dawley female rats	1 minute before death, and also 10 minutes, 4 hours, 12	Samples were divided into 2 groups, namely in open spaces and closed spaces (entomology). The dominant bacteria for estimating post-mortem intervals are in the phyla Proteobacteria, Firmicutes,

		hours, 1 day, 2 days, 3 days, 6 days, and 8 days after death	and Actinobacteria because their numbers change relative to post-mortem time. And the results obtained were that groups in closed spaces experienced a longer decomposition process compared to those in open spaces, which allowed insects to play a role in the process. [10]
Lancu et al (2016)	Intraoral swab of 3 pigs in warm season	Payne classification then divided in 2 times to differentiated the result data	The data results are differentiated based on bacterial taxonomy, the most dominant at the beginning of the decomposition process is the Firmicutes phylum until the 2nd week with the species *Basillus fastidiosus, Clostridium acidisoli, Desulfospira joergensenii, pseudomonas poae* and several bacteria that only appear in the 1st week (*Pseudomonas fragi*) and week 2 (*aclostridium algidicarnis, C. estertheticum, Eubacterium oxidoreducens*) group 2 appeared after week 2 with varying durations of time until week 6. 2 taxa of Gamma-proteobacteria appeared in weeks 3 to 7. The genus most commonly seen is Clostridium with the species *C. saccharobutylicum*. [9]
Lancu et al (2015)	Intraoral swab of 3 pigs in cold season	Payne classification then divided into 3 phase of time	In the initial phase, there were 17 bacterial taxa of the Proteobacteria phylum with the Gamma-proteobacteria class and 1 Beta-proteobacteria class as well as 2 taxa of the Firmicutes phylum, then in the 6th week the Bacteroidetes phylum appeared. In the intermediate phase, it is still dominated by the Proteobacteria phylum with the Gamma-proteobacteria class which then begins to decrease at week 9, while the Firmicutes and Bacteroidetes phyla increase. In the advanced phase, dominance was found by the phyla Proteobacteria and Firmicutes. [11]
Hyde et al (2015)	Swab of left buccal mucosa in 2 cadaver (1 man and 1 female)	Day 1 when the corps start bloating (pre-bloat), bloat, purge, putrefaction and skeletonisation	The dominant bacteria is the phylum Proteobacteria which is then followed by an increase in the phylum Firmicutes. The Enterococcaceae family dominates before the cleaning phase and after the cleaning phase it is dominated by Planococcaceae which then decreases when Clostridium increases. [20]

DISCUSSION

The oral microbiota contains the second highest number of bacteria in the human body, after the gastrointestinal tract, accounting for 26% of the overall microbiota. [7] One area of the body that is simple to access and offers details about a person's general and oral health is the mouth. The changes that occur after death affect the entire human body, including the oral cavity. These modifications can be used as individual features or as attributes of a single occurrence. Changes in the oral microbiota can be produced by physical and chemical changes following death, as well as changes in the ecology that contribute to the decomposition process. This is why some microorganisms can be employed as indicators to estimate postmortem intervals. [19] The existence of oral microbiota anywhere and anytime makes it a viable tool for determining post-mortem intervals, even though the life potential of certain bacteria depends on conditions that can affect the environment.

The estimation of the post-mortem interval by taking changes in the oral microbiota community into account has been studied in a number of research, and the results allow for consideration in this estimation process. According to Dong et al.'s 2019 research, there were results on the relative value of a bacterial community over the postmortem time. The relative values mentioned in the study were classified according to bacterial taxonomy. This study found that Proteobacteria and Firmicutes were the leading phyla during the decomposition phase. [7] These findings are consistent with those obtained by Lancu et al. in 2015 using pig carcass samples during the decomposition process in winter. According to these two investigations, the amount of Proteobacteria bacteria fell at the start of death and subsequently rose as the time interval between deaths increased. [7,11] It is known that a number of bacteria belonging to the phyla Firmicutes and Proteobacteria contribute to the breakdown process that takes place in tissues and organs. [7,21] The findings of this study also support studies by Hyde et al. (2015), which found that the Proteobacteria phylum dominated the oral microbiota following death, with the Firmicutes phylum subsequently showing an increase. This study looked at a number of different body parts as well, and the results showed that the head area went through the decomposition process earliest, with numerous other body areas going through the process at different times. [20] This contrasts with the findings of Lancu et al. (2016), who discovered that the breakdown process happened during the summer. This study discovered that bacteria from the Firmicutes phylum and the Gamma-proteobacteria class predominated. [9] The implication is that the oral microbiota, which is involved in the breakdown process, can be greatly influenced by temperature and humidity levels.

In contrast to the results found in the research of Garriga et al. in 2017 with oral cavity samples from cadavers which were donated for educational purposes. It was found that the bacteria that play a role in the decomposition process at the beginning of death are bacteria from the Firmicutes and Actinobacteria phylum, which are then dominated by the Firmicutes phylum. Until the stage of advanced damage where the oxygen content has decreased, the bacteria of the phylum Proteobacteria and Tenericutes will increase. [8] Guo et al. in 2016 conducted research with various considerations, namely differentiating the oral microbiota in buccal mucosa swab samples of Sprague Dawley rats in open and closed spaces, so that there were differences caused by the presence of insects that played a role in the decomposition process. In this research, it was stated that the decomposition process carried out in an open space or in connection with sacrophagus insects would speed up the decomposition process compared to the decomposition process in a closed space. At the beginning of post-

mortem, both groups were dominated by Proteobacteria which would then change 4 hours after death to be dominated by the Firmicutes phylum in the closed room group and Actinobacteria was the dominant phylum in the open room group. [10] Based on this research, it is stated that environmental and entomological factors play a major role in the decomposition process.

The determination of the post-mortem interval in the research that has been carried out is visually depicting the changes that occur during the decomposition process. In the research of Lancu et al. in 2015 and 2016, Payne's calcification was used, which differentiates the decomposition process into five stages. These stages are not limited in time to hours, days or weeks but depend on the physical changes that occur. The initial stage is characterized by the time when death occurs until the initial post-mortem signs are visible. The next stage is the distension stage which is marked by dilatation in several parts of the body. The next stage is initial damage which is characterized by the release of fluid and gas from the body. This then continues with further damage which is characterized by the tissues starting to dry out and peel off the bones. The final stage is skeletonization where the tissue has dried and completely fallen off. [5,8,9,11] Another study that used a visual basis as an estimate of post-mortem interval was the study of Hyde et al. in 2015 which divided the clinical appearance the same as Payne's classification but with the addition of the initial time of bloat (pre-bloat) which then researchers divided these stages into early post-mortem and late post-mortem. This is different from the research of Dong et al and Guo et al. who use time limits in research. This aims to estimate the post-mortem interval to be specific to a certain time. [7,10]

In the research of Dong et al. Data was obtained on several bacteria that had relative changes during post-mortem. In this study, the results of relative changes were divided based on the taxonomic classification of bacteria. At the phylum level, it was found that Proteobacteria had a value relative to the post-mortem interval where the number of bacteria would decrease in the first 24 hours and then increase with the time of death. Meanwhile, at class level, the relative value is found in Gamma-proteobacteria bacteria. This is in line with the results of the relative values at order and family levels that occur for Enterobacteriales and Enterobacteriaceae where all three increase with time of death. At the genera level, Proteus bacteria experienced an increase after 24 hours after death. These five bacteria are known to belong to the phylum Proteobacteria which is one of the largest phyla in bacterial taxonomy. These five bacteria are gram-negative bacteria with facultative anaerobic metabolism. Proteobacteria are often associated with the process of spoilage in meat and are often found in dead animals. This study suggests that Gamma-proteobacteria and Proteus are potential candidates of oral microbiota communities for post-mortem interval estimation. In this study, the average temperature during the decomposition process was 22.4˚C with an average relative humidity of 37%. [7] In contrast to research conducted by Gariga et al. where the data results show that the dominant bacteria in the cadaver decomposition process in the fresh stage are Firmicutes and Actinobacteria. However, the number decreased until the swelling stage was dominated by Tenericutes, which then at the advanced damage stage was dominated by the Gamma-proteobacteria class. In the final stage, the Firmicutes phylum again dominates, but in the Bacilli and Clostridia classes. This research was conducted at an average temperature of 24.5˚C with an average humidity of 81.16%.[8] In the study, there was no mention of relative values for bacterial composition and bacterial communities that could be used as markers for post-mortem interval estimation.

Research by Guo et al. has an average temperature of 25.74˚C with humidity of 73.2%. The results obtained from the two groups of samples studied. In the first group the samples were placed in a closed room so that

there was no involvement of insects in the decomposition process, while in the second group they were placed in an open room so that insects were involved in the decomposition process (entomology). In this study, the results showed that the phylum Proteobacteria dominated during the decomposition process except at 4 hours post-mortem where the phylum Firmicutes was dominant in the first group and Actinobacteria in the second group. The dominant genera during the decomposition process were Sphingomonas and Streptococcus. In this study, the presence of Proteus and Ignatzschineria bacteria from the Gamma-proteobacteria class was also found. Bacteria in the Gamma-proteobacteria class have different metabolic capabilities and are related to breaking down complex molecules, so this research supports the theory that Gamma-proteobacteria may be important contributors in the decomposition process. [10] In this study, it is stated that the microbiota community can be used as a marker as an additional aid in determining post-mortem intervals.

Research using cadavers was also carried out by Hyde et al. in 2015. In this study, the two cadavers used were donations from special institutions which had previously been frozen at a temperature of -17˚C to -12˚C for 22 and 14 days. The average humidity of both cadavers increased as the study progressed. The microbiota community begins with a large number of Proteobacteria bacteria followed by an increase in Firmicutes. In the oral cavity, bacteria from the Enterococcaceae family dominate before the cleaning stage, which is then dominated by Planococcaceae until Clostridium increases to 47%. It was stated that the weakness of this study was that the sample size was small, meaning it could not determine the relative value of individuality in age, gender or body weight. In general, the two cadavers had variations in bacterial communities before the research was carried out, which makes it possible to show different microbiota during the decomposition process. The cadavers were also frozen before research was carried out, allowing for changes to the initial microbiota. The absence of data on the cadaver's previous history is also one of the shortcomings in this research, so the researchers suggest conducting further research in estimating post-mortem intervals microbiologically. [20]

Subsequent research by Lancu et al. in 2016 which was carried out in the summer for two months. The air temperature at the time of the study varied, from the first seven weeks of the study the average temperature was 25˚C, then in the eighth week it began to decrease with an average of 12˚C until the 13th and 14th weeks had a temperature between 7˚C and 13 ˚C. Relative humidity was between 37 – 84% until the seventh week and reached 73% during the eighth week and constantly increased in weeks 9 – 14. The bacterial community was divided into two time groups. The first group was in the first two weeks and the second group started after the second week. During the decomposition process, the bacterial species Firmicutes and Gamma-proteobacteria dominate with the genus Clostridium. The first group until the second week was dominated by the Firmicutes group (*Bacillus fastidiosus*, *C. acidisoli, Desulfospira joergensenii, Pseudomonas poae*) with several bacteria that were only present in the first week (*P. fragii*) and in the second week (*C. algidicarnis, C. estertheticum, Eubacterium oxidoreducens*). The second group appeared from the second week with varying durations until the sixth week (*Streptococcus porcorum, Clostridium putrefaciens, C. saccharobutylicum*). Two taxa of Gamma-proteobacteria appear in weeks 3 – 7 (*W. Chitiniclastica, I. Indica*). Some bacteria only survived to week 6 (*C. cadaveris*) or only appeared during part of the study (*C. autoethanogenum, Methylomonas methanica, Proteus mirabilis, C. paraputrificum*). Some of the bacteria above are related to fermentation, including *Proteus mirabilis* and Pseudomonas species which are often present in the degradation process. However, Lancu et al. stated that further research is needed to determine bacterial markers from the decomposition process by considering variations in meteorological parameters. [9]

Another study by Lancu et al. in 2015 examined microbiota communities to estimate post-mortem intervals during winter. Still using pig samples, but with an average temperature of 13˚C in the first week and decreasing drastically to -2˚C in the third week. Between weeks 4 – 6 the temperature is relatively stable at 3˚C. which then increased until the 15th week and stabilized at a temperature of 13˚C for the three weeks after that. Week 18 was marked as the warmest week with a temperature of 26˚C until week 22. Humidity was between 46 – 95% and no large variations were found during the research period. In this study, it was divided into three post-mortem times where the initial post-mortem was weeks 0 – 6. Meanwhile, the intermediate post-mortem was at weeks 6 – 17 and the advanced post-mortem was at weeks 17 – 21. At the beginning of the post Mortem revealed 15 taxa from the phylum Proteobacteria and 2 taxa from Firmicutes. The Proteobacteria bacteria that appear come from the Gamma-proteobacteria class and one of them is Beta-proteobacteria. The Gamma-proteobacteria class is dominated by two genera, namely Pseudomonas and Psychrobacter. In the sixth week, the phylum Bacteroidetes appears with one species. Intermediate post-mortem was still dominated by Proteobacteria with five species from the Gamma-proteobacteria class. Throughout the decomposition period, microorganisms from new phyla began to develop in the ninth week, including three new species from the phylum Firmicutes and one species from Bacteroidetes. In the follow-up post-mortem, it was still dominated by Proteobacteria with microorganisms that developed from the Firmicutes phylum. The large number of Proteobacteria bacteria in the oral cavity is said to be because these bacteria are often associated with the decay process. In this study it was stated that the data obtained were not suitable for use as markers in estimating post-mortem intervals. [11]

Based on several studies on changes in the oral microbiota for estimating post-mortem intervals, the exact bacterial markers are still not known. Further research is needed regarding changes in the microbiota in a variety of different parameters, both in terms of temperature and environment.

CONCLUSION

Post-mortem interval estimation is very important in forensic analysis. The use of microbiology to help estimate time of death is one method that has been developing in recent years. Oral microbiota, which also plays a role in the decomposition process, allows community changes to occur so that it can be used as physical evidence for more accurate post-mortem interval estimates. The physical and chemical changes that occur during the decomposition process cause competition and self-defense of the oral microbiota. This is what causes changes in the oral microbiota which can be used as material for post-mortem interval estimation analysis. The microbiota in the human body lives dynamically under normal circumstances, but at the time of death there will be changes and factors that will play a role in the decomposition process. It is possible that these changes will be different for each individual because there are factors that play a role in them. These factors also play a role in the oral cavity ecosystem which will then have an impact on the oral microbiota. The post-mortem interval that is widely used in several studies is using Payne's classification which divides the decomposition process into five stages based on the physical changes that occur. This is said to be more accurate than using a time limit, because each individual is likely to experience a different decomposition process.

Analysis of changes in the oral microbiota also needs to consider the area of the oral cavity that will be used. Because the oral microbiota in each area has different bacterial communities. The buccal mucosa area is an

area that is often swabbed for microbiological research. It is important to know the normal microbiota in a specific area because during the decomposition process the possibility of translocation of non-oral microbiota will occur. So it is necessary to know the normal oral microbiota so that it can be differentiated from microbiota that has translocated from non-oral areas.

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