THE HUMAN MICROBIOME OF SKIN(HANDS) BODILY FLUIDS(ORAL/VAGINAL), AND HAIR(PUBLIC) AND ITS POTENTIAL IN FORENSIC INVESTIGATIONS REGARDING SEXUAL VIOLENCES

By
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To my mother and father for their devotion and support throughout the years
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A special thanks to my beloved parents; Laura and Marlon who without their support in all aspects none of this could be possible. Thanks to Claudia and Charlotte with whom without vast patience and desire to read all my drafts this thesis could not have been possible. Lastly, a heartfelt thanks to my advisor; Dr. Lerah Sutton who always took the time to help me and point me forward.
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<th>Description</th>
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<tr>
<td>HCV</td>
<td>Hepatitis C virus is a viral infection that causes liver inflammation which can sometimes lead to serious liver damage spread via contaminated blood.</td>
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<td>HIV</td>
<td>Human Immunodeficiency virus is a virus that attacks cells that help the body fight infections, making an individual more susceptible to other infections and diseases.</td>
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<td>PMI</td>
<td>Post-mortem interval is defined as the time that has elapsed since a person has died.</td>
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<td>SOP</td>
<td>Standard Operating Procedure is a set of step-by-step instructions compiled by an organization to help workers carry out complex routine operations.</td>
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Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
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THE HUMAN MICROBIOME OF SKIN(HANDS) BODILY FLUIDS(ORAL/VAGINAL),
AND HAIR(PUBLIC) AND ITS POTENTIAL IN FORENSIC INVESTIGATIONS
REGARDING SEXUAL VIOLENCES

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Sexual violence is on the rise with less individuals coming forward to the police, whether that be from shame or distrust in the system (Morgan Ph.D & Oudekerk Ph.D, 2019). Conviction percentages are low regarding cases involving sexual violence typically due to lack of evidence, there is a need to strengthen the chances to be able to correctly identify the culprit. Herein lies the possibility in the future to utilize the human microbiome in conjunction with current DNA analyses to not only identify a culprit but be able to link them to the scene of the crime. DNA is a critical component in sexual violence cases whether or not the human DNA that is retrieved is enough to be amplified and utilized is a major concern for investigators. When human DNA is not enough in sexual offenses and no other evidence is present besides the inconclusive “he said she said” discourse, investigations are typically left unresolved. This is where the human microbiome has great potential to be used in conjunction with traditional DNA analyses in regard to cases involving sexual violence.
CHAPTER 1
INTRODUCTION

The Human Microbiome has the potential to change the way forensic investigators handle cases regarding sexual violence through the bacterial analysis of human touch, saliva and hair. Cases involving sexual violence have shown an increasing trend with cases rising every year with the statistics, unfortunately, indicating that victims have declined. The difficulty in solving these cases surrounds the inability to obtain an adequate amount of evidence to successfully prosecute the suspected assailant. In an attempt to bridge the gap between reported cases and successful prosecutions, I propose to apply the human microbiome, which in this case, refers to the bacteria found on and within the human body, into forensic investigations. Specifically, by utilizing the bacteria found in human bodily fluids and on one’s skin and hair will not only allow investigators to be able to distinguish the presence of distinct individuals from a recovered mixture of fluids from a scene, but might also lead to the ability to identify the assailant from a strand of hair or via one of the articles of clothing they had handled. However, it is important to note that even though this novel idea has many potential attributes, it is a long way from being reliable and valid. The first significant obstacle that must be overcome is the lack of thorough testing done, and the absence of an established SOP, as well as the lack of availability to a database that will encompass the bacterial DNA sequences. The other substantial barrier to this novel idea is that it will likely be some time before the evidence acquired from this methodology is allowed to be entered into court. All evidence that will be presented by an expert witness must go through the Daubert test, this is meant to prevent “bad science” or irrelevant evidence from being used.
Sexual Violence

The CDC defines sexual violence as a sexual activity when consent is not obtained or freely given. The U.S Department of Justice 2018 report on criminal victimization show that there has been an increase in sexual violence crimes from 1.6 to 2.7 victimizations per 1000 persons from 2017 to 2018, while all other forms of violent victimization remained the same statistically e.g., robbery and assaults (Morgan Ph.D & Oudekerk Ph.D, 2019). With present it is noted that 1 in 3 women and 1 in 4 men experience sexual violence. It also depicts that sexual violence begins early with 1 out of 3 females and 1 out of 4 male rape victims experience it for the first time between the ages of 11 to 17 years old. With 1 out of 8 female and 1 out of 4 males at the age of 10 and below. The U.S Department of Justice 2018 report portrays that the percentage of rape or sexual-assault victimizations reported to the police declined from 40 to 25 percent. The 2018 Crime in the U.S report details that of 99,193 sexual offenses only 15,911 were cleared by arrest and 9,652 were cleared by exceptional means. The percentage of those that lead to a conviction is less than 1% based on the accumulated research by the RAINN organization (RAINN, 2017). City Attorney Jon R. Zug gave a presentation on “The Reality of Prosecuting the Sexual Assault Case”, explaining how sexual offenses rape in particular are hard to try mainly due to burden of proof, with typically only two witnesses and at times a lack of trace evidence is it difficult to even try to prosecute an assailant let alone succeed. Sexual violence cases require detailed and prompt collection of trace evidence starting with collection of clothing to collecting hair samples and skin swabs as well (Newton, 2013). The successful retrieval of viable DNA means the possibility of not only identifying the culprit but also a chance of going and succeeding in court.
The Human Microbiome

The diverse microbiota present on the human skin as well within the human body is referred to as the human microbiome. The concept of the human microbiome is not new, Antoine van Leuwenhoek compared his oral and fecal microbiota in the 1960’s yet the term was not coined until 2001. The human microbiota is a collection of up to 10-100 trillion symbiotic microbiotas living on the skin and within individuals (Ursell, et al., 2012). The symbiotic microbiota is composed of not only bacteria but also viruses and single-celled eukaryotes. In short, the bacterial cells present on an individual outnumber the number of human cells in one’s body by ten to one. The research behind this phenomenon is regarded as ‘The Human Microbiome Project’ and with-it researchers hope to understand how it affects our digestive system and immune system (Medicine & NIH). So far the project has been able to determine that some microbes produce vitamins ones body is not capable of creating on its as well as the fact that the human microbiome is highly personalized (Gilbert, et al., 2018 & Ursell, et al., 2012). This entails that each individuals microbiome are distinct, meaning no two human microbiomes are exactly alike even when identical twins are involved (Goodrich, et al., 2914). Diet, lifestyle, antibiotics are the three main factors that can influence ones human microbiome and can thus has the potential to be utilized to determine an individuals geographic location, drug usage, and general health (Gilbert, et al., 2018 & Kapoor & Chowdhry, 2018). It is also important to note that an individual’s microbiome remains relatively stable throughout their lifetime, while antibiotics can alter the bacterial composition of the microbiome it will likely revert back to a state of equilibrium (Caporaso, et al., 2011) & (NIH, 2012).
Microbial Forensics

The original application of ‘microbial forensics’ or ‘forensic microbiology’, began due cases regarding bioterrorism, biocrimes, and epidolomology to trace it back to the source of perpetutor(s), such as was the case regarding the United States Anthrax attack of 2001 where Bacillus anthracis were spread via mail (Schmedes, Sajantila and Budowle & Charaya, Singh and Sidhu). However over the years the application has devled into untilzing the microbial communities located in soil to assist in determing PMI, locating clandestine graves, linking objects that had soil to a localized area (Metcalf, et al., 2017 & Oliveira & Amorim, 2018). PMI has typically been the focus of forensic investigators when the human microbiome is mentioned, with great promise been researched in the reliability in determining PMI (Belk, et al., 2018) & (Pechal, et al., 2018).

Microbial Forensics and Sexual Violence Investigations

The usage of microbiology in cases regarding sexual violence is not entirely novel, tracking of sexually transmitted diseases such as HIV and HCV in cases involving rape, child molestation, and even malpractice cases have been noted (Schmedes, et al., 2016) & (Oliveira & Amorim, 2018). A step father admitted to sexually abusing his stepdaughter once presented with the virological report ordered by the state prosecutor. The report indicated that the step father was the most likely source of infection due to the similarities in the phylogenetic analysis (Banaschak, et al., 2000).

Why Bacterial DNA over Human DNA?

Bacterial cells outnumber human ones with a ratio of 10:1 meaning that these bacteria account for about 1-3% of one’s body mass. An in-depth literature review was done on the estimated numeral count of bacteria researched to be in/on the body
(Sender, et al., 2016). This leads to the suggestion that while human DNA or RNA is recovered yet unusable or if it is not even detectable there is a greater possibility for bacterial DNA to be detected, recovered and usable (Dobay, et al., 2019). Bacterial DNA is better equipped to handle environmental factors than human DNA is and thus has a greater possibility to remain longer on surfaces (Leake, et al., 2013). Another benefit is having the ability to distinguish between bodily fluids, human DNA is human DNA no matter where it is retrieved from unlike bacterial DNA from the human microbiome which in some locations e.g. oral and vaginal has specific bacteria that reside in those areas (Dobay, et al., 2019).
CHAPTER 2
SKIN, BODILY FLUIDS, AND HAIR

In terms of cases regarding sexual violence the most common forms of evidence left behind are physical evidence e.g marks, bruising, bite marks, bodily fluids, and hair. There is sufficient research on the human microbiome that establishes a precedent that the bacterial DNA present on human skin; hands, pubic, and within bodily fluids; oral and vaginal can be utilized to not only accurately identify an individual who committed a crime, but can also provide information of the assailant. (Oliveira & Amorim, 2018).

Skin (Hand)

The main topic of discussion was whether or not bacterial profiling is possible and if it is what are its implications for criminal investigations, specifically sexual violence cases. The microbiome of the skin; including hands which frequently touch various objects, remains stable. A sample period of over 2.5 years was done to prove that a high degree of accuracy was obtained in collecting and attributing these samples to their donor, with the presence/absence of nucleotide diversities in Propionibacterium acnes (Schmedes, et al., 2017). In addition, Park et al., retrieved three specific bacteria that frequent on hands; Staphylococcus, Micrococcus, and Enhydrobacter. With Enhydrobacter being a facultative anaerobe, further testing in anaerobic cultivations would be needed to obtain a clearer picture of its place in the hand microbiome. However, in the 15 samples retrieved both Staphylococcus and Micrococcus was present and due to minor variations in the genetic code verified the ability to distinguish between donors (Park, et al., 2017).
Bacterial Profiling

Typical fingerprinting is most certainly not a novel idea; the earliest usage of ‘fingerprints’ to identify an individual was noted in Chinese culture in 221 to 206 B.C. from the Qin Dynasty (Barnes, 2011). It was not until 1903 where the use of fingerprinting for criminal record purposes was seen in the United States, then in 1911 where fingerprinting became admissible in court and is used in People v Crispi in which a successful conviction was obtained solely on the use of fingerprint evidence (Barnes, 2011). What is proposed by various researchers is to use the bacterial DNA from the skin; hands, to obtain a positive match on the individual it belongs to; this is referred to as “bacterial profiling” (Phan, et al., 2020). Variation across the core set of bacterial taxa from the palm was determined by Fierer et al., which established that different individuals phylotypes shared was about 13% (Fierer, et al., 2008).

Practical Examples

As for practical examples Fierer et al., was able to establish the possibility that bacterial fingerprints could be lifted from an object, in their case, keyboard key and mouse and then properly traced back to the original donor with high probability (Fierer, et al., 2010). In this experiment the researchers were also able to determine that sampling immediately after the touch and up to two hours later yielded no difference in the microbial community of the sample. It also led to the understanding that samples left exposed to typical indoor conditions for two weeks had no influence on the bacterial community, the same result was yielded when the sample was left in a -20°C freezer (Fierer, et al., 2010). Yet, it is important to note only two samples were utilized in this small-scale experiment and additional testing is required to establish an accurate picture. A similar experiment was performed by Lee et al., which swabbed public
computers from a lab and the doorknob to that lab with two individuals who were in the lab. Their research determined that individuals who spend longer periods of time with an object will increase the likelihood of obtaining a positive identity on the individual (Lee, et al., 2015). Cellphones are an everyday accessory which do not leave the sides of most Americans, Meadow et al., was able to determine that individuals' cellphones carry the microbiome of their owners. An 82% similarity was detected and thus exhibiting the ability to distinguish between cellphones and their owners (Meadow, et al., 2014).

**Practical Examples Involving Objects**

Assailants will typically grab their victim to sexually assault them in research done by Lee et al. was able to demonstrate that bacterial DNA from an individual’s palm could be lifted from fabric (Lee, et al., 2016). Not only could a useable amount of bacterial DNA be lifted from the three various fabrics; cotton, polyester, and then a mix of the two, but also the researchers were able to closely match the recovered bacterial DNA to the appropriate donor. Similarly, enough Neckovic et al., set out to determine if the bacterial microbiome could be recovered from paper, cotton, glass surfaces through direct and indirect contact. Their research determined that non-cohabitating individuals could indeed transfer each other's microbiomes via both direct and indirect contact (Neckovic, et al., 2020). In a mock home invasion done in conjunction with the U.S Department of Justice, the investigators were able to detect the “burglar’s” bacterial fingerprint on various objects in the ten homes sampled (Gilbert, et al., 2019).

**Additional Information**

However, if there are not any potential suspects in a case, bacterial DNA from the human microbiome also has the potential to provide a background to the individual. Research done by Phan et al. tested the diversity and abundance of bacteria from
hands and with that they were able to predict gender as well as other personal characteristics such as diet, ethnicity, and even hand sanitizer usage. They were able to determine a 64% accuracy in utilizing the bacteria; *Alloiococcus*, as a method to determine gender, the presence of this bacteria indicating that the donor is male. Similarly, enough in regard to ethnicity the same bacteria; *Alloiococcus*, held a 56% accuracy rate to predict that absence of this bacterium has the potential to indicate that the donor is likely from Asian descent. As for diet, absence of *Lactococcus* held a 48% accuracy rate to determine that an absence of this bacteria meant the donor consumed a primarily Chinese diet. The marker for hand sanitizer usage was also *Alloiococcus*, with a 51% accuracy rate, yet they determined that a larger sample size would be needed to validate their work (Phan, et al., 2020).

**Bodily Fluids (Oral and Vaginal)**

Sexual violence investigations will more often than not include bodily fluids; saliva, vaginal and/or semen either on the victim themself and/or their clothes. When the examination of a victim of sexual violence occurs a mixture of bodily fluids is likely recovered, identification of these fluids can prove some type of sexual contact occurred, recovery and analysis of these fluids has the potential to identify the assailant and provide some background information. Bacterial DNA form bodily fluids remain viable even after exposure to standard indoor conditions across the span of thirty days (Dobay, et al., 2019).

**Saliva**

The usage of saliva in forensic investigations has grown over the past decade, it has been used in cases not only of those of sexual nature but also in animal bite, poisonings, drug/alcohol abuse, and even to identify individuals via human DNA
Human DNA can be retrieved from saliva and is done so in forensic investigations particularly when bite marks are noted. There have been successful attempts to identify individuals not only in laboratory settings, but also in actual investigations (Anzai-Kanto, et al., 2005) & (Sweet & Shutler, 1999). However, recovery of human DNA at crime scenes or on the victim is often insufficient to be useful and the concentration of DNA degrades over time in storage with a significant decrease after five months (Saxena & Kumar, 2015) & (Yong-Woo & Yong-Ku, 2006). Meanwhile the dominant strains of Streptococcus remain stable for long periods of time, with papers showing bacterial detection two years after initial sampling (Bek-Thomsen, et al., 2008). Bacterial DNA retrieved from saliva has the potential to be utilized in conjunction with or separate from human DNA if the amount recovered is deemed insufficient.

**Oral Specific.** Like the bacteria on the skin, the bacteria of the mouth is in somewhat constant contact with the external environment; breathing, eating, etc., yet the bacterial colonies in the mouth remain constant throughout an individual's life (Costello, et al., 2009 & Lazarevic, et al., 2010). Bacteria like Streptococcus are oral specific meaning that it can only be located in the mouth. Researchers Nakanishi et al, were able to determine this by trying to detect either *S. salivarius* or *S. mutans* in semen, urine, vaginal fluid, or on skin surfaces. The two trains of Streptococcus were undetectable in all of the locations, their research also led to the determination that the strain *S. salivarius* could serve as a better marker than the *S. mutans* strain.

**Practical Examples.** The successfulness of *S. salivarius* as the dominant marker was established when the researchers decided to test the ability of recovering
bacterial DNA from cigarette butts and cotton gauzes that were used to wipe off saliva from the skin. Detection for *S. salivarius* strain proved to be close to 100% on both the materials used (Nakanishi, et al., 2009). Leake et al. was able to determine the most appropriate targets to utilize when trying to determine the presence of saliva; a combination of both *rpoB* and 16S rRNA was highly suggested (Leake, et al., 2016). The ability to detect the presence of saliva directly on the skin was determined by Hanssen et al. The experiment entailed the swabbing of dried saliva from fingers with three different collection methods; tape, synthetic swab, and cotton swabs. Their results entailed a lack of reliability with the usage of cotton swabs to retrieve usable bacterial DNA however the synthetic swabs and tape both yielded usable amounts 90 to 60 percent respectively. In their experiment it was also concluded the ability to detect both dry diluted and pure saliva from an individual’s fingers (Hanssen, et al., 2017). With their ability to distinguish between skin and saliva on skin from 135 samples out of 144 illustrates the ability to detect and retrieve dried saliva from a victim of sexual violence and be able to accurately connect it back to the proposed assailant. The ability to accurately differentiate between individuals even those that reported to live similar lives proved to be possible (Wang, et al., 2019). Bite marks on sexual violence victims may be seen and the retrieval of *Streptococcal* DNA has proved to be beneficial. Kennedy et al. was able to connect bite marks back to the owner via bacterial DNA (Kennedy, et al., 2012).

**Vaginal**

Sexual violence may likely include vaginal fluid, detection of this particular fluid is important when a mixture of bodily fluids is recovered at the scene. *Lactobacillus* is predominately found in the vagina specifically the strains (Ravel, et al., 2010). The most
appropriate markers determined were *L. crispatus* and *L. gensenii* with neither being detected in semen, blood or saliva (Fleming & Harbison, 2010). Giampoli et al. was able to properly distinguish between sources of an artificially mixed sample of bodily fluids containing vaginal, anal, and oral fluids (Giampaoli, et al., 2012).

**Practical Examples.** To provide a more accurate and detailed research assessment on the applicability of detecting vaginal fluids in mixed samples with the use of ForFLUID kit Giampoli et al had four homogenous vaginal samples processed by eight different laboratories. All eight of the laboratories had very consistent and similar results (Giampaoli, et al., 2014). The ability to detect vaginal fluid can also assist forensic investigators in determining if any type of objects were used to sexually assault the victim.

**Hair (Pubic)**

In forensic investigations hair is typically found at crime scenes due to the fact humans are constantly shedding their hair. The first reported use of hair-comparison for forensic investigative reasonings was in 1861 by Rudolf Virchow; typically regarded as “the father of modern pathology” (Oien, 2009). One of the issues with hair analysis is the for extraction of DNA root hairs are recommended and yield the highest and accurate results (Hue & Linh, 2013) yet typically when recovered from crimes scenes roots are not attached and thus will yield lower if any amount of DNA (Magalhães, et al., 2015 & Opel, et al., 2008). Another compounding issue is the fact that pubic hair transmission during sexual intercourse occurs only about 17.3% of the time; with male to female transmission at 10.9% and female to male at 23.6% (Exiline, et al., 1998).
Practical Examples

These issues lower the likelihood of identifying the assailant, yet with the microbiome found in the pubic hair researchers have detected that the microbiome within the pubic region is distinct between individuals. The benefit of utilizing the bacterial DNA from pubic hair is that it is stable and is not as influenced by the environment as scalp hairs are (Tridico, et al., 2014). Tridico et al. performed bacterial profiling on human hair; scalp and pubic over the course of five months with a total of forty two samples. Their first discovery was the clear distinction between male and female pubic hair bacteria, with the absence indicating that the hair belongs to a male (Tridico, et al., 2014). Another note of discovery was the transferring off microbiota during sexual intercourse, two of their participants had had sexual intercourse eighteen hours prior to collection with their samples indicating a mixture of taxa different than the previous collections (Tridico, et al., 2014). Adding onto the research performed by Tridico et al., Williams and Gibson did a similar study involving twelve couples and nineteen singles to compile microbiome profiles from their pubic hair and swabs from their pubic mound region. Their research set out to confirm whether or not sexual contact between individual could be detected via pubic mound region microbiome transfer. While the couples did reveal a variation within their samples as was in the research mentioned above, it was noted how there is minimal research on the detection of microbiome transfer in single sexual encounters (Williams & Gibson, 2019). This was based upon the fact that in order to detect a microbiome transfer at least 10% of the attacker’s pubic microbiome must be derived from the victim (Williams & Gibson, 2019).
CHAPTER 3
APPLICATIONS

The ability to utilize the human microbiome in forensic investigations is an entirely novel idea and prior to it being able to be used in day-to-day investigations it is critical for it to be rigorously tested. My proposition is for this method to undergo a three step process for its development before it is applied in conjunction with other current investigative practices. The first application will ensure that there is indeed groundwork and that it will be a useful method for investigators. The second application will test the consistency and accuracy of this method. Lastly, the final application will determine if this method can hold its own against current investigative practices.

First Application

The first step would be to conduct a mock crime scene. Going through the typical motions of collecting evidence from the scene and victim then processing the samples to determine if any bacterial samples can be retrieved. If there are samples that differ from that of the victim, then the following step would be to assemble a pool of suspects which includes the assailant to then be able to conclude if any matches; correct or incorrect occur. Doing this with various types of sexual violence cases will provide a foundation for whether or not this method can be useful in these specific types of investigations.

Second Application

Before this method can be utilized in ongoing forensic investigations and at the risk of encumbering the department's resources, I propose a team of investigators and lab technicians go back to older cases that have an identified assailant and retest the available evidence to determine if this method is successful. The first action would be to
conclude if bacterial DNA could be lifted from any of the collected evidence items. If doing so is possible the next step would be to determine its origin, whether it is pubic and oral bacteria then the next step would be to compare it to a pool of suspects including the known assailant and see if any matches occur. This of course would need to be repeated a various amount of times with different cases and with the different types of collected evidence, clothes, swabs, etc.

**Third Application**

The final course of action that will determine the true validity of this method would be to use alongside ongoing investigations. If an individual allows the medical professional to collect evidence after an incident, a sexual assault kit will be used. This entails swabs, test tubes, microscopic slides, and collection of hairs and fibers (Justice, 2020). Investigators should be able utilize the same collection method and preparation method done for human DNA for bacterial DNA. The differentiation would be in the amplification process which is simply using primers designed to amplify bacteria DNA instead of human one to be able to identify what kind of bacteria it is and obtain its unique code. Utilizing both human and bacterial DNA, allows for investigators to not solely rely on bacterial DNA and start to establish the validity of the method. At the minimum, investigators would be able to determine that an individual did indeed bite the victim or penetrate her and still have that assailant’s unique bacterial code and would be able to compare it to any assailant that is suspected.
CHAPTER 4
CAVEATS AND LIMITATIONS

Bacterial profiling has the upmost potential to become a new method in assisting forensic investigators in identifying the assailant. Yet, the research suggested in the chapters above are novel and has only been performed under controlled laboratory settings. There are also several questions that must be answered prior to being utilized in investigations. As previously mentioned, there is still much rigorous testing to be performed yet there are other items that must be dealt with prior to allowing this method to stand on its own not only to properly assist in an investigation but can be held up in court.

Caveats

While the researchers presented above have proven their hypothesis to be true; “the human microbiome can serve as a method of identifying individuals”, it is critical to note that additional and in-depth research is required before it can be utilized in forensic investigations. For example, the collection methods used are similar to those performed in an investigation, as sterile as possible with little outside contamination. Yet, the methods used to recover bacterial DNA varied between what kits were used to what computer program was utilized to display the results. There is a need to create a general outline of how collection must be performed, what kit to use depending on the evidence, and what program is best utilized. Many of these projects also had a limited sample size with one solely having fifteen sample.

Limitations

A major setback which can be readily solved is the lack of SOPs for this new methodology. The methods mentioned above used to recover bacterial DNA differed as
did what kits and computer programs were utilized to obtain their results. A major issue that must be resolved prior to determining the validity of the methodology would be to set up a database to store all the bacterial genome sequences as well as an algorithm that would be able to note the difference in the sequence and make note of it and be able to compare it to other sequences entered for a match. This database would also have to enable a formula that can differentiate between the microbiotas, e.g. skin vs. oral and then log them correctly. Another question that should be answered when constructing the database is if two samples from different microbiota are recovered and belong to the same individual can they be connected and if so, how will it be done. Furthermore, even if all these complexities are resolved it becomes a moot point if investigators are not able to stand up in court to provide expert testimony in order to establish the connection between the victim and the assailant with this new method. In the United States there are regulations and guidelines in place to prevent untested and erroneous methodologies from being entered into court. This testing is regarded as the Daubert test which is utilized to determine an expert’s testimony is admissible in court (Daubert v. Merrell Dow Pharmaceuticals, Inc., 1993). For this to occur five statements must be stated as true:

1. The theory is testable.
2. That the theory has been peer reviewed.
3. The reliability and error rate.
4. The existence and maintencase of standards controlling its operation.
5. The extent of general acceptance by the scientific community.

Once these five points can be proven an expert witness will then be able to exhibit their
findings in court. Additionally, bringing up the issue of who can and will be classified as an expert witness once this method is ‘tried and true’, curriculum and/or additional training must also be developed. While it is certain that there is an abundant amount of research to be done it is necessary to ensure the potential and legitimacy of this new methodology.
CHAPTER 5
CONCLUSIONS

Cases regarding sexual assault are some of the most difficult to try, being that there are rarely any witnesses to the attack, and that evidence is, at times, sparse and lackluster. With sexual assaults cases on the rise and to bridge the gap between reported cases and culprits who are sentenced, I propose to continue researching the ability to utilize the human microbiome to assist in solving sexually violent investigations. The research done thus far on the bacterial profiling of the microbiome of the skin, saliva, and pubic hair has established the precedent that it is indeed possible to identify an individual based on their unique microbiota from distinct locations. In the mock crime scene performed by the U.S Department of Justice highlights the potential in obtaining bacterial fingerprints from objects touched by the assailant or in their case the faux burglar. As for bodily fluids, the research done in on the bacterial composition found in this specific area illustrates how beneficial bacterial DNA is and its ability to degrader slower and last longer in the environment allowing investigators greater opportunity to be able lift and process this bacterium and be able to connect is back to the assailant. With the minimal research performed on vaginal bodily fluids, researchers were able to distinguish this particular fluid type when present in a mixture. In an investigative setting uncovering unknown fluids at a scene is common and while it definitely will not state that there had been an assault it can assist in illustrating that there had been some type of sexual act involved, allowing for a better understanding of what occurred. In reference to pubic hair while its utilization in forensic investigations is not a novel idea, the utilization of the microbiota found in pubic hair is indeed novel. While, the ability to detect the transfer of pubic microbiome has not been explicitly
definitive with the research implemented thus far, however the ability to distinguish between male and female pubic hair has been determined. Again, potentially providing the investigators with another piece of evidence that may not have been explicitly clear at the scene beforehand.

The research completed thus far has certainly established a precedent into the great potential this novel idea could have not only in sexually based forensic investigations, but in forensic investigations as a whole. The work performed by the researchers mentioned above is in no means a complete collection; on the contrary, it's merely a glimpse of its potential which is why it must be further developed and continued to be heavily tested to determine the efficacy of this method. Once the reliability of this method is determined it will be critical for investigators to then be able to apply these methods to different scenarios to confirm its practicality has to be during real time forensic investigations. Such models include utilizing mock crime scenes and re-testing evidence from previously solved crimes. There is also a need for various SOPs’ to be properly developed, which will allow researchers and investigators to have a methodical way to prove the accuracy and validity of this methodology before it can be used as a standalone technique. While the ability to have this novel method be used either in conjunction or as a standalone is important it is as critical that the results be allowed into court as admissible evidence. For this to occur, the Daubert standard must be applied and must ensure that the four criteria previously mentioned are achieved.

My goal is that this accumulation of research will ease the passage of future researchers and investigators who want to continue to explore this methodology by assisting them in acquiring grants and funding to be able to continue to develop this
new technique for future general acceptance. With the hopes of in the future being able to easily obtain otherwise arduous evidence that can assist in providing justice and even comfort to victims of sexual violence and their families. While also narrowing the ever-growing gap between reported and sentenced cases, allowing for these victims to feel a sense of comfort and confidence to report their assault. This new method will also add another tool to the metaphorical toolbelt investigators have with them when investigating a case, potentially providing them with comfort as well knowing they have another technique to use to ensure there is enough evidence to convict. The future research and studies on the human microbiome may also prove beneficial to mankind as well revealing new information about our own bodies that otherwise may have taken longer to be discovered and utilized. I believe that the results from my research support my hypothesis; while there is still much work to be done before its inclusion into current investigative practices, there is undeniably a foundation for researchers and investigators to strengthen and build upon.
LIST OF REFERENCES


Sender, R., Fuchs, S., & Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLOS Biology, 14*(8).


BIOGRAPHICAL SKETCH

Laura Montesano has a Bachelor of Science with a major of microbiology from the University of Florida. She obtained a Master of Science with a concentration of forensic medicine from the University of Florida College of Medicine in 2020.