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Eastern Kentucky University

Catching the Perfect Serial Killer: How Bacterial Evidence Can be Used to Advance a

Forensic Investigation

Honors Thesis Submitted In Partial Fulfillment Of the Requirements of HON 420 Fall 2018

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Catching the Perfect Serial Killer: How Bacterial Evidence Can Advance a Forensic Investigation

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Abstract: Catching the Perfect Serial Killer: How Bacterial Evidence Can Advance a *Forensic Investigation* is a short story and process statement that serves to bring attention to the forensic possibilities that the field application of bacterial evidence presents. This project also allows individuals not familiar with the field to have a better understanding as to how bacterial evidence could be used to link a perpetrator to their crime. The short story, Catching the Perfect Serial Killer, follows Professor Amanda Newman at the University of California. A serial killer in her neighborhood leaves no forensic evidence behind. When the FBI takes over the case, they come to her for help. She uses bacterial cells left behind by the killer to help catch him. But, before the killer is caught, he goes after Amanda. The FBI must use the bacterial evidence to find the killer before it's too late. The process statement, How Bacterial Evidence Can Advance a Forensic Investigation, explores what bacterial evidence is, how it can be analyzed, and how it can advance a forensic investigation. The process statement also explains why bacterial evidence can be used as a legitimate forensic tool by addressing the leading concerns for using bacterial evidence in a forensic investigation.

Keywords: Forensic Investigation, Bacteria, Short Story, Evidence, Honors Thesis

ii

Title Page	i
Abstract	ii
Table of Contents	
Acknowledgements	iv
Catching the Perfect Serial Killer	1
Prologue	2
Chapter 1	4
Chapter 2	9
Chapter 3	11
Chapter 4	14
Chapter 5	19
Chapter 6	22
Chapter 7	25
Chapter 8	
How Bacterial Evidence Can Advance a Forensic Investigation	33
Bibliography	43

Table of Contents

Acknowledgements

I would like to gratefully acknowledge various people who have journeyed with me this past year as I have worked on this thesis. First, I would like to thank my mentor, Dr. Bill Staddon, who has helped me through the thesis process. I found inspiration for my story through his guidance.

Secondly, I would like to thank all the Honors faculty for their help and guidance throughout my college experience. I have learned so much through my Honors' courses. I have so many precious memories that I will cherish for the rest of my life.

Lastly, I would like to thank my friends, Katie Clark and Brooke Russell, who I owe an enormous debt of gratitude for their support. They have gotten me through some stressful times this past year. I will forever be thankful that they are my friends because they are the best people anyone can know.



CATCHING THE PERFECT SERIAL KILLER

By Catherine Wood



Prologue

Friday night is a time to relax after a week spent at work. This Friday night Stacey Lewis was at a bar with her friends, letting off some steam, and drinking. Stacey Lewis should not have left the bar alone. She should not have drunk so much that she could barely walk straight. She should not have done a lot of things. Unfortunately, she left the bar drunk and alone. Her only thoughts were about making it home and sleeping it off. She only lived a couple blocks away. What could go wrong?

Stacey stumbled home. She didn't notice anyone following her. She opened the front door, not bothering to lock it behind her. She went straight upstairs to her bedroom. She took off her shoes and fell into bed. She passed out as soon as her head hit the bed.

As Stacey left the bar, a man took notice of her, or more importantly, who wasn't with her. He saw that she was drunk and alone. He smiled and started following her in his car. She stumbled most of the way, but she soon arrived at her house. He noticed none of the lights were on, meaning she lived alone. *Perfect*, he thinks. He parked the car down the street. The man got out and grabbed his bag. Making sure no one was looking, he walked back to the house to hide behind a bush. He waited until it appeared she was asleep.

The man got prepared. He put on gloves, a mask and a hat and went to the front door, testing to see if it was locked. It wasn't. He removed his shoes and put it in his bag before he entered the house. As quietly as possible, he moved around the first floor, taking in the possibilities. He went into the kitchen, looking for a knife. Grabbing the sharpest one he could find, he quietly went upstairs. He found her bedroom easily. He slowly opened the door, not letting it make a sound. He set his bag down and got out a syringe. He crept across the floor to the bed. He looked down at the sleeping woman. He reached down to caress her hair and push it away from her face. He then plunged the needle into her neck, releasing the paralytic into her system.

A sharp pain in her neck woke Stacey, but she couldn't move. Suddenly, she saw a face. She tried to scream but couldn't. The face smiled at her. The last thing Stacey saw was her knife in his hand.

Stacey Lewis didn't show up to work on Monday. That was not like her. Her friend was worried. At the end of the day, when Stacey still didn't show up at work, her friend decided to go to Stacey's house. The door opened when she knocked on it. All the lights were off. She felt a cold chill go down her spine. She knew in that moment that something was wrong. She quickly went up the stairs, calling Stacey's name. Stacey's bedroom door was ajar. She pushed the door open, and what she saw made her scream.

"...The Night Killer struck again last night. Police say the killer entered the victim's home and stabbed the owner 36 times with the owner's knife. Last night's victim brings the count to 11. Police still do not have any suspects. Let's go live to our reporter on the scene. Tom..."

Professor Amanda Newman turned off the tv as she was getting ready for work. She didn't want to think about the killer that attacks close to her neighborhood. She wanted to focus on her commute to work. The University of California in San Francisco was a perilous commute if you tended to space out while travelling it. She is currently having trouble with her research. As a microbiologist, she deals with everything to do with bacteria. There is so much people can learn from bacteria. But, right now, she needs to learn how to get to the university without crashing.

Leaving her neighborhood in San Carlos becomes problematic. Police are everywhere. It takes her 20 minutes before the police allow people through. It takes another hour because of traffic, and by the time she gets to her office, she is late for her class. She quickly gathers her materials and rushes to the classroom. The classroom itself is not a huge lecture hall, but it's not small either. Her advanced microbiology class only has 20 students, but she prefers smaller classes so that she can talk more individually to her students.

The chatter quiets as she enters the room saying, "I'm very sorry for being late. Let me get this set up and we'll begin." Amanda plugs in her laptop and tries to display her PowerPoint on the projector. "We left off last time discussing the human microbiome. What can you tell me about this?" She asks the class.

A student raises his hand, "It's the bacteria that are on our skin."

Amanda nods, "Anyone else?"

Another student raises her hand, "Aren't they also in us? Like, they're microorganisms that are in and on us."

"Very good Molly. Yes, specifically they are symbiotic microorganisms that are found in us and on our skin. Now, can anybody tell me why these symbiotic microorganisms matter?" Amanda was met with silence. "No? Then let's learn more about it. Everyone, turn to page 182. You'll notice some interesting facts on the page."

Molly raises her hand again, "Ma'am, this says that the symbiont ecology is important to the host's health and development. What does that mean?"

Amanda smiles, "That's a good question Molly. Would anybody like to answer that?" Again, silence. "Then I want everyone to read this chapter and discuss the answer with the person sitting next to you. While you do that, I'm going to see if I can get this projector to work."

While she attempted to get it working, a message popped up on her screen. It was an alert from the police. She clicked it open and read the message: *This message is being sent on behalf of the San Carlos Police Department. The San Carlos Police Department has asked the residents of San Carlos and surrounding cities to be on alert at night for suspicious individuals lingering outside of gathering places. Always travel in groups. If* you see suspicious activity, call 911 immediately. It is advised that residents not be out after midnight.

Amanda feels her heart start beating faster in her chest and feels stomach drop. She thinks about the house that had the police blockade this morning. *I wonder if that's where the serial killer struck last night*, she thinks. Her thoughts are interrupted when a student clears his throat.

"Ugh, ma'am? Is everything okay? You went pale."

Amanda mentally shakes herself, "Yes, I'm fine. Just remembered something I need but forgot at home. Now, since I can't get this thing to work, why don't we go over what you were reading? Who can answer Molly's question? Why is symbiont ecology important to the host's health and development? Rebecca, can you give me an answer?"

Rebecca cleared her throat, "Well, symbiont ecology is how the microorganisms interact with us. So, that is important because that interaction can be good or bad."

"Very good. Would anyone else like to elaborate on what Rebecca said?" A student raises his hand, "Go ahead John."

John looks at his book, "This says that some hosts can be dependent on their symbiont, like the symbiont provides some sort of function for the host. So, I think this is saying that we rely on the microorganisms to keep us healthy, which helps us to develop."

Amanda smiles, "That is exactly right, John. What is unique about the human microbiome?"

John raises his hand, "Aren't they unique to every person, like fingerprints?" Two men in suits enter the class.

Amanda nods, "Specifically, because there are as many as ten times the number of bacterial cells in our body as human cells, the differing types of sub-species of bacteria is unique to everyone. Every person develops their own unique community of bacteria. So, twins will not have the same human microbiome. Can anybody tell me if the microbiome changes?" Silence. "Why don't you guys research that and give me your response next class? I want no more than a paragraph. Have a good rest of your day."

Everyone starts to pack up. The men in suits come up to Amanda but seem to wait until everyone is gone before speaking. Amanda's stomach drops again, "What can I help you with?"

The man closest to her responds first, "Are you Professor Amanda Newman?" She nods yes. "I am Special Agent Rhodes. This is my partner, Special Agent Wilson. We are with the FBI. We are wondering if you have time for a few questions."

Amanda gathers her materials, "I can give you a half an hour. Can you walk with me to my office? I have to get the next class's materials." Rhodes nods and moves to open the door for her.

As the agents follow her, Amanda asks, "What questions do you have?"

Rhodes responds, "Have you seen the news about the Night Killer?"

Amanda stops walking and turns to face the agents, "I have. He's been killing in San Carlos. I live there. Are you here to see if I am a witness?" Wilson shakes his head no, "No ma'am. We are here to see if you would lend a hand with the investigation. We are taking over for the police."

Amanda is shocked, "What do you think I can do to help? Don't you have forensics?" Rhodes looks around at the students staring at them, "Why don't we continue this in your office?"

Amanda nods and continues walking towards her office. Meanwhile, John Barnes, or The Night Killer as the news has dubbed him, sleeps peacefully in his bed. After cleaning up and making sure no trace of him was left behind, John went home to relive the night in his dreams. His alarm wakes him up. He sighs happily as he gets up. John Barnes loves his job as a janitor for the University. It gives him the flexibility he needs to continue his nightly activities. He also likes to listen to the professors talk about their subjects, especially the forensic professors. *They give good pointers for how to not leave any forensic evidence*, he thinks. But, there is one professor that John likes to talk to: Professor Amanda Newman.

Amanda unlocks the door to her office. The agents follow her into her office and close the door. Amanda drops her materials on her desk and cleans off the papers on the chairs. She gestures for the agents to sit down as she moves to her chair behind the desk. Rhodes clears his throat, "This serial killer leaves no trace of himself. Our profilers say that he is in his thirties. All that we know about him is how he kills. He uses a paralytic on his victims so that they can't move as he stabs them with a knife the victim owns. The profile says that the stabbing means that he has intimacy issues, but his victims look different."

Amanda interrupts, "What is it you want from me?"

Wilson leans forward in his chair, "We want to know more about your bacteria research. Specifically, can it help catch this guy? One of our forensic people said you could help." Amanda leaned on the desk, "Well, I have been doing some research into the human microbiome, since it is unique for every person. While the microbiome does change in terms of the proportions of sub-species of bacteria, the overall makeup of the community does not. However, the little changes in the microbiome could prove useful to you."

Rhodes, intrigued, asks, "How so?"

Amanda leans back in her chair, "My research shows that those little changes could tell you information about the host's lifestyle. So, if you get me a sample of bacteria from the killer, then I might be able to tell you details like his job or what medicines he takes. There's bound to be some bacterial cells on the knife or in the crime scene." "Why would you think that?" asks Wilson.

"We shed around 30 million bacterial cells every hour. Think of it like trace evidence. Your killer walks into your crime scene, not knowing that he is shedding bacteria as he goes. You said he used a knife. That knife will have his bacteria on it, even if he used gloves. His gloves will have his bacterial residue that he then transfers to the knife."

"If we were able to get this sample, could you run it and tell us who he is?" asks Rhodes. Amanda shakes her head no, "I might be able to tell you what kind of job he has, but not his name. I might be able to help you narrow him down. I could potentially be able to match samples if you find a suspect. I must say that all this is still under research. You will have a hard time getting this admitted in court if it comes to that."

Rhodes smiles, "We appreciate your help, Professor. If we get you that sample, could you run it for us?" Amanda nods yes. "Then," Rhodes and Wilson stand up. "Here's my card. I'll give you a call when we have something. We won't take up any more of your time today."

Amanda gets up as well, "Let me give you my card." She moves from the desk and hands Rhodes her card. Rhodes says, "Thank you," and the agents leave her office. She looks at her watch. *Great, now I have eight minutes to get to my next class*. Amanda grabs her things for her next class and rushes towards it, not seeing John Barnes staring.

Amanda dismissed her last class for the day. As she was gathering her things her research student, Katie Prichard, came up to her. Katie has been helping conduct some of Amanda's research into bacteria as part of her college credit. Amanda liked Katie. She gets good results.

"Professor Newman, you live in San Carlos, right?" asks Katie.

Amanda smiles, "I do. Why do you ask?"

Katie looks to see if anyone is listening before asking, "Did you get a message from the police? I live near there."

"I did. Are you worried about the serial killer?"

Katie nods her head, "I am. I was wondering if I could leave the lab early tonight, so I can be home before 9. My roommates are staying with other people more outside of the city."

Amanda hopes she can reassure her, "Of course you can leave early. I was going to make leave early myself. Do you have someone to stay with?"

"I am from out of state, so no. And my boyfriend is in Ireland currently."

"Okay. Here's what we'll do. I'm going to give you my phone number. I want you to call, or text, me if you don't feel safe. Does that sound okay?"

Katie smiles, relieved, "Thank you so much Professor Newman! You are the best."

Amanda hands her phone number to Katie, saying, "You are very welcome, Katie. Was there anything else?"

"No. Thank you again and see you tonight!" Katie smiles and leaves the classroom.

Amanda sighs once Katie is gone. *I hope the FBI will catch this guy soon*. Amanda herself feels uneasy about being home alone, but she feels a little better now that the FBI has taken over. While she is contemplating things, a janitor enters the classroom, whistling. Amanda smiles, recognizing him as John Barnes. Amanda always takes the time to talk with him. *He's nice*, she thought.

John stops whistling, "Hello Doc. How did your class go?"

Amanda smiles, "It was fine, John. Thanks. How's work going? Did Marvin leave his samples in the trash again?"

John laughs, "He did. Luckily, I'm here to make sure they don't actually get thrown away."

"Marvin and this university is very lucky that you're here."

John smiles, "Thanks, Doc. That means a lot to me. Did you know that you're the only person to actually speak to me here?"

Amanda is shocked, "Really? I'm sorry."

"Don't be, Doc. I'm just a janitor. I'm not a Professor like you."

Amanda smiles sadly, "Still. I'm sorry on their behalf. Now, tell me how your date went the other night."

"Oh. It went alright. We just went out for drinks. She had a little too much, so I helped her home, but I don't think we'll go out again." John internally smiles, remembering last night. John saw on the news today that his 'date' was named Stacey.

"That's too bad. But, at least you are going out on dates. That's more than what I'm doing currently," says Amanda, not meeting John's eyes.

John goes up to Amanda and places his hand on top of hers, "Don't worry, Doc. There's someone out there for you. It might even be the next person you see."

Amanda laughed, "We'll see. Thank you though, John. That was sweet of you. Now, I'm off. Have a nice rest of your day."

"You too, Doc," John smiles as Amanda leaves. *She'll see soon enough that I'm the man for her. I just need to show her that I'm the man for her*, John thought, his smile dropping from his face.

Amanda enters her lab to see Katie already set up. Amanda wants to run some tests on profiling the human microbiome considering her visit from the FBI. She wants to run her samples and try to see if she can match them. If she can do this consistently and accurately, she might be able to help catch the killer.

"Alright, Katie, we are going to deviate from our original plan today. What do you know about sequencing the 16s RNA gene?"

"Um, that is used to study the different taxa of bacteria, right?"

"Yes. Why would we use this for sequencing the human microbiome?"

"Because it is a genetic marker that is present in almost all bacteria. It also identifies the bacteria genus. So, it tells you what's there."

Amanda smiles, "Very good, Katie. Now, what about Metagenomics?"

"Well, metagenomics utilizes shotgun amplification sequencing. It makes copies of all the DNA in the sample and sequences as much of it as possible. It can tell you about the bacterial community, like if their host takes medicine."

"Excellent. Today we are going to be doing both. Since you are more familiar with the 16s RNA sequencing, I want you to run these samples and analyze them. I'm going to work on the Metagenomics on another sample."

Amanda handed Katie two samples. What Katie didn't know was that the samples contained Amanda's microbiome. Amanda was going to run the shotgun amplification

sequencing on her microbiome to see how accurate it is. *If this works, then I'll have more confidence in what I tell the FBI*, she thought.

Meanwhile, the CSI unit for the FBI was collecting the samples from all over the crime scene at Stacey Lewis' house. They collected swabs from the door handles and kitchen. They also swabbed the knife on Professor Newman's advice. All the swabs were logged and sent back to the forensic laboratory on the lead investigator's order. After everything was done, Special Agent Rhodes dialed Professor Newman's phone number. Amanda picks up, "Hello?"

"Hello. This is Special Agent Rhodes. We spoke earlier. My forensic team has just collected some samples were the killers bacteria could be. It will take a day for us to process this evidence into our system. Are you available to come down to our lab in two days?"

"Is there any way that I could analyze that evidence in my lab? I have all the machines and supplies necessary to perform the analysis."

"That can be arranged, but we would need to ensure that the chain of custody is not broken. I would need to post a couple of agents outside of the lab, and you would need to be alone. Is that agreeable?"

"If it helps catch this guy, then that would be fine."

"Okay, then I'll call you tomorrow for a time. Have a nice night, Professor."

"Good night, Special Agent Rhodes." Rhodes hung up.

Back at the lab, Amanda and Katie are almost done getting everything ready to be sequenced. Amanda had stepped out of the lab to answer her phone. When she comes back, Katie is cleaning up her work station.

Katie looks up when Amanda walks in, "Everything is ready. I took the liberty of loading my samples into the machine. All that needs to be done is to hit start when you are ready."

Amanda puts her phone away, "Thank you, Katie. Since you're done, why don't you head home? I'll finish up and head out. We'll analyze the results tomorrow."

Katie smiles, "Thank you Professor Newman." Katie gathers her stuff.

"Katie, remember to call if you need anything. And why don't you text me when you get home?"

Katie opens to door, "I will. Thanks again. Bye and have a good night."

"Bye, Katie. Drive safe," Amanda says as Katie leaves.

Amanda goes back to what she was doing. All that's left is to load her samples into the machine as well. She loads them and starts the sequencer. She then starts Katie's sequencer. The results will take a while, so Amanda will leave them to run overnight. She is cleaning up her workstation when John enters the lab.

John looks at her and smiles, "Working late again, Doc?"

"Just finishing up, actually. How many rooms do you have left?"

John empties the trash, "This is my last. I'm heading out after I get done."

"Well isn't that a coincidence? Looks like we're both leaving. Got any plans?"

John shakes his head, "No, ma'am. I'm just going home. I might catch up on some shows. How about you?"

Amanda also shakes her head, "Sadly, no. I'm going home to read some more research articles." Amanda gathers her stuff as John finishes up.

John opens the door as they leave the lab, "Do you want company on the way to your car?"

Amanda smiles, "That would be lovely, John. Do you need to do anything else before we leave?"

"I just need to clock out. It will only take a minute. Do you mind waiting?" Amanda shakes her head no. They walk to the janitor's station. John clocks out. Then they're heading towards the employee lot. "Do you live far away?" John asks.

"I live about 50 minutes depending on traffic."

"That's the same for me. Do you by any chance live in San Carlos?"

Surprised, Amanda asks, "I do. How did you know?"

"That's where I live. How about that. We live in the same city and didn't know it."

"It sure is something. Did you get that message from the police?"

John nodded, "I did, but I'm not too worried. It seems that the killer is only after women. It's awful that the police haven't caught him yet. Are you worried?" "I'm less worried than my research student. She is currently alone in her house. I told her to call me when she gets home."

"That's really nice of you to look after her like that."

They come to Amanda's car, "This is me. And if I can help ease her concerns just a little bit by doing that, then it's no hardship. Thanks for walking me to my car, John."

"It was my pleasure, Doc. Drive safe and be careful. See you tomorrow." Before Amanda gets into her car, she reaches up and give John a kiss on the cheek. Then she gets in her car and drives off, leaving John stunned. *Soon*, he thought.

The next day saw Amanda and Katie analyzing their results. Katie was able to match the samples given to her to each other. Amanda's results showed that she mostly works in a lab, diets, doesn't take any medications, and works as a teacher. The results were all accurate. Having research that backs up what she said to the FBI eases her nerves. Now, she can run the samples they give her and be confident in the results. Amanda was about to tell Katie she could leave when her phone started ringing. It was the same phone number Agent Rhodes called her from yesterday.

Amanda answered, "This is Professor Newman."

Special Agent Rhodes answers, "This is Rhodes. I am calling to set a time for tomorrow. What will work best for you?"

Amanda thinks about her schedule tomorrow, "How about 3 o'clock? And, can you make sure that I have a reference sample from the victim. It will ensure that I am giving you the killer's lifestyle."

"It has already been done. We'll meet you at your lab then. Have a good day, Professor." "Thank you, Agent Rhodes. You too." Amanda ended the call.

Katie looked at Amanda, "Do we not have research tomorrow?"

"It looks like you have a free day tomorrow. I'm going to be helping the FBI with some bacterial evidence they have."

"That's so cool! Can I help?"

"I'm sorry, Katie, but I have to do it myself. Until you get your degree, I am the only one qualified to handle the analysis."

"Okay, but can you tell me anything about what you're going to be doing?"

Amanda nodded, "Let's just say it will be like last night. Also, you shouldn't worry so much about your living situation."

Katie looked hopeful, "Really?"

Amanda smiled at her, "Yes, but I still want you to text me when you're home. Don't hesitate to call me either." Katie smiled and nodded. "Okay, then that will be all today. Have a good weekend."

"Thank you, Professor. Good luck!" Katie left the lab.

Amanda spent the rest of her time getting everything ready for tomorrow. She made sure every reagent and material she could use wasn't expired. She also made sure she had extras just in case. She set aside some room for the evidence. After everything looked in order, she set the trashcan outside the lab for John and locked the room. She left a note telling John not to go into the lab. She went to her office to gather some papers and left to go home.

What Amanda didn't know was that John wasn't collecting trash that night. He saw that Amanda was leaving early. He decided to follow her home. *Just to make sure she didn't have a date*, he thought. Amanda didn't leave her house after she got home. That made John happy. It wasn't until the last light went out that John decided to go

home. He had something special planned for Amanda. *Soon she will see me as her someone special*.

John lived a couple of streets from Amanda. It surprised him when he found that out. He had a crush on the nice Professor, but he was too shy to ask her out. But, it was late, and he wanted to make sure nothing bad happened to her. *There are a lot of crazy and bad people at* night, he thought. So, he offered to walk her to her car. He was elated when she said yes. They parted ways. They were more than halfway home before he noticed that he kept seeing her car. He was curious, so he decided to see how far away they lived from each other. He was happy when it was only a couple of streets away.

The next day, John was going to tell Amanda about his discovery when someone ran into her, knocking the papers she had to the ground. The student didn't notice and continued walking. John ran up to her, knelt, and helped her pickup her papers. The moment she looked up and smiled at him is the moment he decided she was going to be his. He just had to prove himself to her. He had to prove that he was a man. *I know the perfect way*.

Amanda unlocked her lab the next day 15 minutes before the evidence was supposed to arrive. She went in and made sure everything was still in place. It was, so she went around turning on all the machines she needed. Now it was time to wait. She only had to wait another five before there was a knock on the door. She went to open it and saw four agents carrying various bags of evidence.

"Oh my! Did you collect everything in the house?" Amanda exclaimed.

"Sorry, ma'am. Where can we put these?" one of the agents asked.

"Right. You can place those over there," she said, gesturing to the cleared off space. The agents set the bags on the table.

"Sorry about all the bags, Professor. I told the CSI's to collect anything that could have the killer's bacteria. Each bag is marked with where it was collected. And this," Agent Rhodes says as he hands Amanda a swab, "is your reference sample from the victim."

"Thank you, Agent Rhodes. Any suggestions on where to start?"

"I suggest you start with the knife. Everything else is up to you. I'm going to leave a couple of agents outside the lab to make sure it's secure. Let them know if you need anything."

Amanda nodded, "Okay. Depending on how long it takes to go through these samples, I should have some results tomorrow."

"Thank you, Professor. Agents Abner and Harris will be stationed outside. Good luck." Agent Rhodes left Amanda to go through the evidence. Amanda catalogued the evidence and gathered everything she needed for it to be sequenced. She is going to sequence to 16s RNA gene to determine what bacteria is present and if it belongs to the victim or not. She is also going to run the shotgun amplification sequencing on the samples that do not match to the victim. *Hopefully, I'll be able to help the FBI narrow down who the killer is.*

Amanda prepared all the samples first for the 16s RNA gene. After they were ready, she loaded them into the sequencer. She looked at her watch and noticed that several hours had passed. She decided to go get dinner. She left the sequencer running while she left her lab. She smiled at the agents and asked if they wanted anything. They nodded no. She went to her office to eat the lunch she forgot earlier. As she was eating, John knocked on the door. He smiled at her as he collected the trash.

"Working late again, Doc?"

"Yeah. I'm helping someone sort through some bacterial samples."

"Is that why there are two scary looking guys outside your lab door?"

Amanda leaned forward conspiratorially, "Yeah. Between you and me, I'm helping the FBI with a case."

John looked surprised, "Wow. That's amazing. Looks like someone is moved to the big leagues."

Amanda laughed, "Yeah, except they gave me a lot of evidence to sift through. It's going to take me the rest of the day to get it done."

"Well, then you better enjoy your dinner. It looks like it will be the only break you get."

"Thanks John. You're real helpful. Oh. You'll have to tell one of the agents when you want the trash. No one's supposed to go into the lab but me and the agents." John left the office saying, "Will do, doc. Have a good night."

"Thanks. You too, John." Amanda finished eating and went back to the lab.

Amanda started to put everything back in order. She had to wait for the results to know which samples to run the metagenomics on. She didn't have to wait too much longer, luckily. Most of the samples belonged to the victim, but there were several that possibly belonged to the killer, including the knife. She prepared those samples for the shotgun amplification sequencer. She then placed them in the sequencer and let it run. It was nearing 11 pm when she looked at her watch. She cleaned up everything and noticed the trash was gone. She turned off the lights and locked the lab.

Looking at the agents she asks, "Do you guys go home, or do you have to stay here?"

Abner smiled, "Shift change is at midnight, ma'am. The evidence must be secure, but we don't have to stay here all night."

"Well, that's good. Have a good night, guys."

"You too ma'am," Harris says.

Amanda left the building, tired after a long day. She didn't notice John get into his car to follow her home. She also didn't notice that he stayed outside until she turned off her lights.

Amanda arrived at her lab early in the morning to go over the results. What she found disturbed her. There were many similarities to her own results. She immediately called Agent Rhodes.

"Special Agent Rhodes," he answered.

"It's Professor Newman. I've got some results for you. If you want to come to my lab, I would like to show you something."

"I'll be there as soon as I can, Professor." Amanda ended the call.

Agent Rhodes showed up 20 minutes later, "What have you got for me, Professor?"

"I eliminated majority of the samples because they were from the victim. Now, the others were from an unknown person, possibly the killer because they match to what I found on the knife. Based on the samples you gave me and the concentration of the bacteria, I can walk you through what this person did in the house."

Rhodes interrupts, "How can you tell that?"

"We shed around 30 million bacterial cells into our vicinity every hour. So, a greater concentration of bacterial cells is indicative that the person spent a greater period with an object or in a position."

"Okay. Tell me what he did."

"Your people were very thorough and swabbed almost everything. This person spent some time with the outside door handle. Once he entered, he went around the first floor before going into the kitchen. There, he stood in front of the knives for a longer period. Once this person grabbed the murder weapon, they went upstairs. This person didn't spend much time looking for the bedroom. Once inside, the person spent majority of their time near the victim. Then he retraced his steps, which I assume was to clean up after himself."

Rhodes looked impressed, "Wow. That's exactly what we theorized he did. What else were you able to tell about him?"

Amanda sighs, "That's why I wanted you to come here. I need to show you this." She points to a side by side screen showing the results for the test she ran on herself and the evidence. "What does this show you?"

"Well, I'm no expert, but they look very similar," Rhodes says.

"Right. The one on the left is a test I ran on myself. The one on the left is from the evidence."

"What does that mean exactly?"

"It means that the person lives in San Carlos. That's where I live. This person also eats very similar to me. I'm too busy to remember to eat half the time, so I grab fast food on my way home. I can tell you that this person is a janitor who is on some antidepressants and antipsychotics."

"It makes sense that if this person lived where you do that they would get the same food," Rhodes reasons.

"Yes, but mostly only fast food? That's not a normal diet."

"That is true. So, we are possibly looking for a male in his 30s who works as a janitor. He is on antidepressants and antipsychotics and mostly easts fast food. Anything else?"

Amanda shakes her head no, "Not that I can tell at this time."

"Okay. Then we'll get all of this out of your way. I would like to say again thanks for your help. I will personally keep you updated on the case."

"Thank you, Agent Rhodes."

Rhodes nods and tells the agents to take the evidence back to the forensic laboratory. Amanda is left alone in her lab thinking that the profile sounds a lot like John. *It can't be him. He is so sweet and wouldn't hurt a fly!* She thinks. She makes sure to leave before John does his trash collection. By the time she gets home, Agent Rhodes lets her know that they have narrowed down the list of suspects and asks if she would be willing to match reference samples. She tells him yes before getting out of the car. She doesn't hear John come up behind her until he injects her. Her world turns to darkness.

Amanda slowly came to. The first thing she noticed was John smiling down at her. Then she noticed that her hands were tied to her dining room chair. The table was set for a romantic dinner for two. The lights were turned down low and candles were lit all over the room. Amanda's stomach dropped as realization hit that John was The Night Killer. She opened her mouth to speak, but her mouth was too dry.

"Here, let me help," said John, holding a glass of water out for her to drink through a straw. "What do you think? Is it too much?" John was gesturing around the room.

Amanda decided to play along, "I think it's lovely, but why am I tied up?"

"Well, I didn't want you to ruin it by leaving."

Amanda smiled at him, "Now why would I leave when you've gone to all this trouble for me?"

"Really? You really like it?" John asks hopefully.

"I do. Why don't you untie me, so I can enjoy it fully," John still looks doubtful, "How about you just untie my arm?" John nods and unties her arm. He goes into the kitchen.

Amanda quickly thinks about how she is going to get out of this. *Clearly his antipsychotics aren't helping*, she thinks. She looks around the table for something to cut her restraints with but finds nothing she can reach. She's not left with many options. She tries to get the phone from her pocket. It's hard with one hand, but she manages to call her last number, Agent Rhodes. She can only hope that he answers the phone and listens as she quickly puts her phone on silent and back in her pocket. John returns from the kitchen carrying plates of food, "I made dinner for us. I hope everything is to your liking. I've been planning this night for months."

Amanda smiles at him, "I'm sure it will be delicious. Why didn't you say anything before now?"

John looks down at his feet, "I was too shy. I wanted to make sure that you couldn't say no to me before I told you." John puts food on her plate. It's basic. Mashed potatoes and Mac 'N Cheese with microwavable Salisbury steak.

"What did you do, John?" Amanda asks, certain of the answer.

John looks at her and smiles, "I proved I am a man. You deserve a real man, so I made myself into one."

"What exactly did you do, John?"

"I'm The Night Killer, which is a cool name by the way. I needed to be a man and take control. So, I took control of those girls. It was very empowering. It gave me the courage I needed for tonight. I can now be the man you deserve."

Amanda held back the vomit that rose in her mouth. "I'm not sure all that was needed. I would have gone out with you if you had asked."

John looked angry, "Does that mean you don't like what I've done for you? I've worked really hard to be the perfect man for you!" John was shouting now.

"No! I love what you've done for me! This is the most anyone has ever done for me.

Thank you for this special night." This seems to have calmed him down.

John smiles at her, "Good. I'm glad to hear that. Why don't we eat?"

Amanda nods in agreement. They start eating. The food is still a little cold. She doesn't want to make him madder. So, she eats all her food. They don't talk while they eat. Amanda is still trying to think about how she will get John to let her go, when he sets down his knife and fork and gets up. He quickly goes to the window. Amanda's hopes rise. John mumbles under his breath. He then quickly runs to the kitchen. Amanda hears someone knocking on the door.

"Jonathan Barnes, this is the FBI! Come out with your hands up. We don't want any harm to come to Professor Newman," Amanda recognizes the voice as Special Agent Rhodes.

John comes running out of the kitchen with one of her knives, "We are having a special night. Leave us alone! You are not taking her away from me," John shouts.

Amanda quickly says, "John. They will come in here and take me if you don't make them understand that I want to be here. They have to see that I'm okay."

John stands still for a moment before he moves toward her with the knife. Amanda only has a moment to panic before the door is kicked in by the FBI outside. Agent Rhodes sees John advancing toward Amanda with the knife and fires on him. Amanda shouts as he falls on her. Agent Rhodes quickly moves John off Amanda and checks her for injuries.

"Are you okay? Did he cut you anywhere?" Agent Rhodes asks as he unties Amanda from the chair. Amanda, shocked, doesn't speak. "Professor!" Rhodes shouts in the hopes that it gets through to her. Amanda jumps. "Are you okay?" Amanda nods weakly, "I think so. He-he injected me with something that knocked me out."

"Okay, we're going to get you checked out at the hospital. I want you to go with this paramedic. I'll meet you there after I'm done here. Will you be okay until then?"

Amanda nodded and left with the paramedic. Amanda came out of her shocked state when they reached the hospital. She was immediately led to an examination room where a nurse took her stats and blood. They ran a test to see if anything was still in Amanda's system. Her results came back clean. The nurses left her alone for a while. Amanda wasn't sure how long she sat there before Agent Rhodes showed up.

"The doctor says that you're going to be okay, physically. Are you up for giving me your statement."

Amanda nodded and started recounting her night. By the time she got to the end, she was shaking. Agent Rhodes came to her and put his hand on her shoulder. She flinched but didn't shake off his hand.

"I want you to know that none of this is your fault. I mean it. He might have been obsessed with you, but anything he did does not land on you. It was your help that led us to him."

Amanda perked up at that, "Really? Did you get my call?"

"Yes. We were on our way to his house when I got your call. It didn't take us long to change course. Because of you, we were able to get him before he killed anybody else." Amanda felt the tension leave her, "Thank you for telling me that." "No, Amanda, thank you. Your analysis solved this case. This city owes you a debt."

Agent Rhodes left. Amanda was released the next day. She made a resolution to herself as she was checking into a hotel. She was going to dedicate her research towards using bacterial to help forensic investigations. She doesn't want an investigation to be without the added benefit of bacteria.

The End

How Bacterial Evidence Can Advance a Forensic Investigation

By Catherine Wood

Mentor

Dr. Bill Staddon Department of Biological Sciences In Forensics, bacteria are mainly analyzed in response to a biocrime or bioterrorism event. This is known as Microbial Forensics, which is associated with using bacterial evidence to trace the origins of different strains of biological hazards (Kaur). However, this field does not use bacterial evidence to serve regular forensic investigations. A normal forensic investigation involves evidence that can link a perpetrator to their crime, like deoxyribonucleic acid (DNA) and fingerprints. Bacteria are not used, but there are so many ways in which bacteria could be used to advance a forensic investigation.

Identifying and matching suspects is very important in forensics. Any type of evidence that can lead to an identification is crucial. DNA and fingerprints are the most common evidence that are used to identify and match suspects. However, DNA evidence cannot always be reliable. Modern forensics have the power to profile DNA from very small samples. Secondary transfer is a potential risk when collecting these samples. DNA can be transferred between individuals, causing contamination (Cale, 2015).

There have been cases where a person's DNA have been found on or near victims when that person had nothing to do with the crime. A case in 2013 involved the accidental transfer of a person's DNA to a victim via a paramedic. A paramedic had taken Lukis Anderson to the hospital before responding to the murder of the victim. The paramedic had accidentally transferred Anderson's DNA to the victim. As a result, Anderson spent months in jail, charged with the murder of a person he had never met (Cale, 2015).

It's cases like Anderson's that call for the need to have evidence that can add to the confidence that a person committed a crime. Bacterial evidence can add to that confidence. However, the application of bacterial evidence to improve a forensic

investigation comes with concerns. Leading concerns about this forensic method include validity, court approval, standardization, power of discrimination, accessibility, and the ability to link a perpetrator to a crime (Mannucci, 2018).

It is important in a forensic investigation that all results are valid, meaning that the chance of the results being incorrect is very small. Invalid results could send the wrong person to jail, or they could let the guilty free because the method used was not an accepted analytical method. The field application of bacterial evidence faces concerns of validity because it is a relatively new field in forensics. There are theories that need to be explored and proved before they can be validated as a forensic method of analysis. The problem is finding a way to bridge the gap between bacterial evidence and approved techniques already in use in forensic laboratories.

Microbiome profiling could potentially serve as a compliment to human DNA profiling because the bacterial taxa, especially at the subspecies level, appear to be unique to each person, just like DNA. Bacterial (or microbial) DNA can be sequenced using terminal restriction fragment length polymorphism (TRFLP). Microbial DNA can be analyzed using this technique because the fragments are analyzed using the equipment currently available in forensic laboratories. The TRFLP technique basically generates a fingerprint of an unknown microbial community. Amplification of a target gene is performed by tagging primers with fluorescent dye. Then, restriction enzymes are used to cut fragments of the targeted gene. Each bacterium will be cut in different places with the restriction enzyme. The fragments are then separated in a DNA sequencer, which is available in forensic laboratories. Then, the fragments are detected using a fluorescence detector in the lab. Only the fragments with the dye will be read. The different sized fragments make up the identity of the microbial community (Heath, 2006).

TRFLP is used to sequence the 16S RNA gene. Bacteria contain circular chromosomes made of DNA in the cell. The 16S RNA gene is present in almost every species of bacteria, and it is also unique between species. Sequencing the 16S RNA gene produces a specific arrangement of A's, T's C's and G's. Each species of bacteria can be identified by their sequence from the 16S RNA gene. A database could be created that compliments the Combined DNA Index System (CODIS), which is the one used in DNA profiling. All the sequences from the bacteria can be put into a database for comparison. For instance, if you have soil on a shovel that you suspect to be from a crime scene, then you can profile the DNA and match them using the microbial DNA database. The soil sample's microbial DNA would be compared to the known microbial DNA in the database. One would be able to match and identify the unknown sample. Analyzing the microbial DNA and creating a database could become an effective method for lowering crime rates and clearing cases (Heath, 2006).

The most important concern facing the field application of bacterial evidence is court approval. The courts, namely judges, are hesitant to allow new forensic methods to be used in court. When DNA analysis was first introduced as evidence, the court system would not allow it to be used (Shewale, 2014). Judges want techniques that are perceived as a valid method, like polymerase chain reaction (PCR). The biggest problem is getting the court system to accept any bacterial analysis presented to them. Acceptance is only one aspect of the problem. The bacterial analysis method used must also withstand any appeals brought forth after trial. The appeal process is much stricter than the initial process of acceptance.

The Supreme Court set the standard for what can be admitted in court through *Daubert v. Merrell Dow Pharmaceuticals, Inc.* in 1993. The court case identified four factors that a forensic scientist must satisfy in order to be an expert witness in court. Judges can decide which of the four factors must be met: (1) whether the science can be been tested again; (2) whether the science has been published or peer reviewed; (3) whether there is a known error rate and is acceptable; and (4) whether the science has been accepted by the relevant general scientific community. Using techniques that have already been accepted by judges will help bacterial evidence be used in court (Mannucci, 2018).

Power of discrimination is a concern because it is essential to the field of forensic science. Power of discrimination refers to the uniqueness of a sample, meaning that there is an infinitesimal chance that a sample could be match to two different people. For instance, the power of discrimination of DNA needs to be high, as in the DNA of a person has a 1 in trillion chance of matching to another person. More study needs to be done to make sure the power of discrimination of bacterial evidence is high enough to ensure that each sample could only come from one person. Having this assurance will help to allow bacterial evidence to be presented in court.

One way to ensure that a sample could only come from one person is to analyze the human microbiome. The human microbiota is a collection of microorganisms that are found on or within us, like on our skin. These microorganisms include, among others, bacteria. The human microbiome specifically refers to the genetic make-up of the microorganisms. The microorganisms form a symbiotic relationship with their host. They co-exist without harming them. The human microbiome is also unique for everyone. It is estimated that there are as many as ten times the number of bacterial cells in our body as human cells. This means that there are many different sub-species of bacteria. Every person develops their own unique community of bacteria that contains different sub-species. Even twins will not have the same microbiome. This is important for analysis because it is estimated that we shed around 30 million bacterial cells per hour. This leaves a lot of evidence behind that can identify an individual (Hampton-Marcell, 2017).

Whole Metagenome Shotgun Sequencing (WMSS) is a method to analyze the human microbiome. This method utilizes Metagenomics, which is the study of microbial communities from sequenced DNA. WMSS breaks down a chromosome that is too large to be sequenced. The whole chromosome, containing DNA, is cut into different fragments that can be sequenced. The smaller DNA strands are sequenced. The individual sequences are assembled, in order, into the overall sequence (Eisen, 2007).

This is done by looking at the areas of the fragments that overlap. These areas tell you what order the fragments go in. Once the order is determined, then one can assemble the whole sequence of the chromosome. This process is then repeated several times. Software is used to read and identify the microbial communities from the sequences. From there, the community can be profiled. The communities affect and respond to their environment. The bacteria in and on us form a symbiotic relationship with us, their host. How they interact to what we do, and where we go allows us to predict the relationship the bacteria have with our microbiome. This means that bacteria can help predict their

host's lifestyle characteristics, like their diet and occupation, using the relationship they have with their host (Eisen, 2007).

Another leading concern for the field application of bacterial evidence is the standardization of how to analyze to evidence. PCR could be standardized because DNA has the same basic structure for every person, but the bases varies per person. For bacterial evidence, there are different kinds of bacteria that can be analyzed. People have their personal human microbiome, but they also pick up different strands of bacteria that is not associated with their human microbiome. For each type of bacterial evidence, a standard method of analysis needs to be established to gets results validated. One standard method is more reliable then several methods because it decreases the chance for error.

The human microbiome could also help determine the post-mortem interval, which is the time that has passed since a person has died. In forensics, accurately estimating the time since death is critical. It establishes a timeline of the crime, which can lead to suspects. The typical way that the post-mortem interval is estimated is through looking at the stages of decomposition. However, it is hard for the people observing the stages of decomposition to be consistent with other observers because the process of decay is a continuous and dynamic process. Other evaluation methods have been developed, but each come with limitations (Hauther, 2015).

The human microbiome offers an alternative. Specifically, the human gut microbiome plays a role in breaking down soft tissues after we die. After death, our cells break down, releasing substrates that the microbes eat. The microbes in the gut then ferments cellular byproducts into gases. This causes the bloating that is observed during decomposition. So, changes in decomposition lead to a sequence of changes for different populations of microbes (Hauther, 2015).

The changes can lead to a decline in abundance of some populations over time. What this means is that quantifying the abundance of certain microbial populations can accurately estimate the time since death. Over time, bacteria disappear at different rates when you die. One can determine the time of death by looking at the steady rate of decline. This rate gives you can equation to use. That equation is used to find the time that has elapsed since death (Hauther, 2015).

Using this method to determine the time of death is more reliable and accurate than any other method. This process can also be standardized, allowing it to be the one method that does not rely on people's observations. People are not infallible. They make mistakes. This method quantifies the bacteria. It does not allow room for people to make mistakes.

Having processes that are available in most forensic laboratories are important because it means that different types of samples could be run in a single lab. Not one sample would require a specific machine to analyze it. Specialized equipment would not be available at every laboratory because they would cost too much. Being able to analyze bacterial evidence using machines already in laboratories will allow the field application to be more readily accepted by the forensic community. This also saves on cost because there will not be a need to create new machines to analyze the bacterial evidence.

The ability of evidence to link a perpetrator to a crime is why forensics is such an important part of an investigation. The linkage is what proves a person was either at the

crime scene or committed the crime. A person's DNA could be left at a crime scene, and that sample could be matched to a specific individual, proving without a reasonable doubt that the person is guilty. This ability is essential for the field application of bacterial evidence. If the analysis of evidence does not have this ability, then it is useless to the investigation and court system.

The scholarly article, "Tracking Plant, Fungal, and Bacterial DNA in Honey Specimens," presents a case study that explores the feasibility of using PCR to accurately identify honey's microbial components. The study concluded that PCR could be used to analyze bacterial DNA. Because PCR is widely used in forensics already, using PCR to amplify extracted bacterial DNA would be more widely accepted in the forensic community and court system. This case study also illuminates how bacterial DNA could be sequenced, using PCR, to link a perpetrator to a crime. This case study also explains some of the pitfalls associated with using bacterial DNA instead of human DNA, while also suggesting ways those pitfalls could be overcome (Olivieri, 2012).

The scholarly article, "The Earth Microbiome Project: Successes and Aspirations," addresses the concern about what primers to use for bacterial DNA because PCR needs be run using the correct primers for each sample. A study arose to find a primer that allows *Pelagibacter* to be amplified. In Microbial Forensics, if the correct primer could be made to run PCR on bacteria found at a crime scene, then a perpetrator could be identified more easily because bacteria does not degrade as easily as DNA does. This would be an extraordinary step in advancing the field of Forensics (Gilbert, 2014).

The most significant scholarly article, "Methods in Microbial Forensics," introduces microbial forensics as a subfield of forensics, arguing that the field of microbial forensics

has been implemented to enable better confidence in the scientific analysis of forensic evidence and in the interpretations of the results obtained. According to the article, the sample collection and prevention, the extraction of target for analysis, the analytical component of the process, and the interpretation of the results is essential to making sure that the processes used are airtight, validating the field application of bacterial evidence (Kaur, 2014).

These methods for analyzing bacteria all could advance a forensic investigation. There might be some situations where DNA or fingerprints won't be enough to identify a suspect. Or, having extra bacterial evidence could add to the evidence already collected. It will only help a forensic investigation. In order for bacterial evidence to help a forensic investigation, the concerns about validity, court approval, standardization, power of discrimination, accessibility, and the ability to link a perpetrator to a crime need to be discussed and dealt with. Using methods that are already in practice in forensic laboratories to analyze bacteria will help deal with the concerns.

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