The Investigation of Oral Pathogens Associated With Neurodegenerative and Cardiovascular Disease and The Link to Periodontal Disease

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Introduction

Periodontal diseases cause infections and inflammation of the gums and bone that surround and support the teeth [1]. There are four stages of periodontal disease, the first being gingivitis [2]. Gingivitis is a common and mild form of periodontal disease that causes irritation, redness, and swelling to one's gingiva, the part of the gum around the base of the tooth [3]. If untreated gingivitis can develop into periodontitis a more serious form of gum disease [3]. In early periodontitis there is slight bone loss of bone that supports the teeth, this is the second stage of periodontal disease [2]. In the third stage of periodontal disease, moderate periodontitis causes more bone and gum tissue loss which can lead to the loosening of teeth [2]. The final and most advanced stage of periodontal disease is advanced periodontitis where bone and gum tissue loss has caused teeth to become very loose making biting and chewing painful [2]. If one has a periodontal disease that is not diagnosed by a dentist, or is continually neglected after a diagnosis; this can allow for the periodontal disease to progress into another stage becoming more severe and dangerous. The consequence of this neglect is oftentimes the loss of a tooth, or teeth, but may also include other severe underlying consequences.

In a study that was conducted by the Centers of Disease Control (CDC) in 2012, 47.2% of American adults who are above the age of thirty showed some form of periodontal disease [1]. In a 2009 study that was published in the *Journal of Dental Research*, 64.7 million Americans showed signs of mild to severe periodontitis. The study also showed that the rate of infection and classification of severity increased as the age of the demographic increased, as 70.1% of patients aged sixty-five years or older showed signs of periodontitis [1].

Prior studies have placed heavy emphasis on a group of bacteria that are highly associated with periodontal disease: *The Red Complex*. This subgingival micro-complex encompasses the following three pathogens: *Porohyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia.* However, these few species of oral pathogens are not alone in their contribution to periodontal disease. In an ecosystem that consists of over 700 microbes, the mouth is colonized by various other species that could potentially contribute to the formation and progression of periodontal diseases [4].

Current research in the dental field has shown promising results that could possibly prove a link between periodontal disease and cardiovascular disease. However, the current studies investigating a possible link between periodontal disease and neurodegeneration have shown to be heterogeneous.

Throughout this investigation, the goal was to determine whether there could be a potential link between periodontal disease and neurodegenerative or cardiovascular diseases, while also gaining a better understanding of the oral ecosystem. The primary focus of this experiment was not focused on *Red Complex* bacteria, but rather other bacteria found in the oral flora that could contribute to the progression of periodontal disease or cause various forms of cardiovascular disease or neurodegeneration as a result of periodontal disease.

It was hypothesized that there would be a presence of bacterial species in normal oral flora that have been associated with cardiovascular disease and potentially neurodegeneration that could also be linked with periodontal disease.

Methods

Before samples could be collected, research was conducted to determine the best media that would promote optimal growth. In a 2013 study, which evaluated the synergistic polymicrobial biofilm development between *Porphyromonas gingivalis* and *Treponema denticola*, suggested three different growth media for Red Complex bacteria: brain heart infusion (BHI), oral bacterial growth medium (OBGM), and tryptic soy broth (TSB) [5]. For the purpose of this study, a tryptic soy broth media was used because it was a generic media that could be made in large quantities in the lab.

Four variations of the tryptic soy broth media were created for the purpose of introducing different nutrients to each sample.

The neutral media with no nutritional enrichment would serve as a control measurement in all sample comparisons. Two separate plates would consist solely of either Ritz cracker or Domino pure sugar cane. Ritz cracker was chosen as it was a simple form of a carbohydrate that also had added sugars and sodium. The final plate would consolidate the Ritz cracker and Domino sugar cane, and was done to emulate a nutrient environment that bacteria may be accustomed to in the oral ecosystem.

In a 2020 article published by Colgate, it was noted that recent studies have suggested that certain sugars play an important role in the development of oral biofilm, such as plaque, and specifically with oral *streptococci* species [6]. It was decided that the Domino pure sugar cane would be replaced by the three sugars outlined in the article: glucose, lactose, and sucrose. Glucose was not readily available in the lab, so dextrose was used. Dextrose was used as it was nearly chemically identical to glucose found in the bloodstream.

Once an appropriate amount of nutrient plates were made and sterilized in the autoclave, subjects were acquired around the lab and one researcher's home to gather saliva samples. The subject's identity would remain confidential, only their extracurricular habits were recorded for data purposes (i.e. use of tobacco or electronic cigarettes). Once all information was recorded, subjects were given mouth swabs, along with a cup of saline. The mouth swab was submerged into the saline to ensure sterilization. Once the swab was submerged for no less than five seconds, subjects were instructed to take general sabs of their mouth (approximately four cycles around the mouth). Salival samples were aseptically z-streak onto the desired nutrient enriched environment. To avoid contamination of samples, subjects were required to use one swab per sample, and dispose of wasted swabs appropriately.

As the experiment progressed, one subject was sampled in specific regions of the mouth, which were anterior facial tooth surfaces and third molar buccal regions of the mouth. The purpose of this specified swab was to compare the differences between micro-communities between these two specified regions to the general mouth swabs; which would potentially give insight as to whether specific regions showed better growth, and if various species demonstrated adhesion to specific surfaces.

Once samples were successfully transferred onto enriched media plates, they were incubated at varying conditions based upon temperature and oxygen supply. These independent variables determined whether certain species of bacteria may be temperature sensitive or obligate anaerobes. The objective was to evenly separate each set of samples between these four conditions. The two temperatures tested were 37℃ (normal body temperature) and 30℃ (room temperature). Oxygen supply was varied between anaerobic and aerobic environments. The anaerobic environment was used through an EZ GasPak apparatus, which removed oxygen from a concealed environment. The aerobic environment was created by simply incubating samples in the appropriate temperature environment with no concealment or oxygen removal process.

Each plate was allotted approximately four days to grow in its designated incubator. Once the growth period ended, plates were removed from their incubators and evaluated for various growth sites of interest. These sites were

marked on their original growth plates and isolated into individual tubes that would contain liquid broth using a sterile pipette tip. The isolation tubes were set in an agitating incubator at their designated temperatures and oxygen presence. To create an anaerobic environment with the isolation tubes, the caps of each tube were sealed tight, while the aerobic tubes were left unscrewed and held together with a piece of tape. All tubes were in incubation for four days. Once the second round of incubation concluded, another round of evaluation was conducted, and was followed with DNA extraction. By following QIAGEN's DNeasy ® UltraClean ® Microbial Kit Quick-Start Protocol, DNA could successfully be extracted from each desired sample. Once DNA was successfully extracted, each sample was run through a polymerase chain reaction (PCR), which would exponentially increase the DNA present in the sample by a factor of two. Following this reaction, gel electrophoresis was conducted in order to determine whether the sample had yielded enough growth before being sent for DNA sequencing. The gel was a solution of 1g agarose and 100mL TAE buffer (tris base, acetic acid, and EDTA). Positive and negative electrodes were used to expand the DNA band for approximately forty-five minutes at 102 EVT across the agarose gel. A ladder was present in each gel (shown far left of each gel) to determine length of each band in kilobase pairs (kbp). The final stage of preparation before sequencing could occur was the purification of DNA. QIAGEN's QIAquick PCR Purification Kit protocol was used for this process.

Before the extraction phase began, it was decided to duplicate each sample in the experiment. This was done so that in the case of an error occurring, another sample with the same DNA template would be available. These pairs of matching DNA were consolidated into one tube prior to the PCR purification process.

With purified DNA ready to be sequenced, each sample was assigned a specific SimpleSeq Tube, which was provided by Eurofins Genomics, Inc. This lab would sequence a section of 16s Ribosomal DNA, and in return would give information pertaining to chromatogram data, as well as an alignment graph.

By using the chromatogram data from each sample, the DNA sequence could be run through a nucleotide Basic Local Alignment Search Tool (BLASTn). Once the sequence was processed in preference to a desired similarity, the samples would be matched with various species of bacteria that were present in the database of the National Center for Biotechnology Information (NCBI).

Once samples were successfully identified, characterization could begin. Research was done on each identified sample on the genus and species level. The characterization of each species focused primarily on virulency, pathogency, and resistivity. Extensive research was conducted on each species' ability to become associated with any cardiovascular disease or any form of cognitive decline.

Additionally, several identified bacterial species would be tested to examine individual performances against varying antibiotics and oral hygiene agents.

The final post-sequencing experiment that was briefly conducted was the examination of various species of bacteria together in a nutrient enriched environment. Qualitative measurements were recorded following a four day incubation period at 37℃ .

All plated samples were preserved in a 4℃ freezer until further experimentation is conducted.

Results

The first two weeks of experimentation served to be the proof of concept phase. Through trial and error, varying conditions were tested to ensure that bacterial samples could grow under the desired conditions.

It was discovered within the initial two-week proof of concept phase that, although an anaerobic environment yielded growth for the initial week, the environment became nonviable in the subsequent week. To avoid further complications in the later stages of the experiment, and to stay on track for the following progression of the project, it was decided to solely

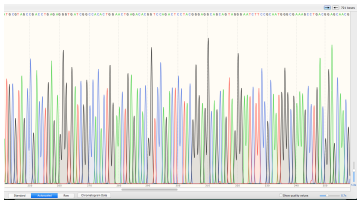
focus on facultative anaerobes that could be found in an aerobic environment.

Throughout the allotted ten-week research period, 58 samples were collected amongst 6 subjects. Subjects varied demographically based on ethnicity, age, consumption of alcohol, dietary habits, and tobacco usage. Each subject admitted to consistent upkeep of oral hygiene. This was determined through gel electrophoresis, which was conducted prior to DNA sequencing.

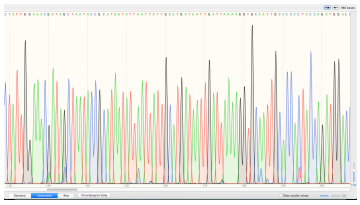
Once Sanger sequencing was completed by Eurofins Genomics, Inc. and chromatogram data was identified through a nucleotide Basic Local Alignment Search Tool (BLASTn), species could successfully and accurately be identified. Of the fifty-eight samples collected, thirty-eight samples were successfully sequenced, giving a sixty-six percent yield. Of the thirty-eight successfully sequenced samples, nineteen samples were identified under the *Staphylococcus* genus, which was approximately fifty percent of the sample yield. The *Bacillus* genus was the second most frequent genus that was identified, with eleven samples, or twenty-nine percent of the yield. Five samples showed the presence of a *Rothia* species, which made up thirteen percent of the yield. Two *Streptococcus* species were sequenced, making up roughly five percent of the sample yield, and one *Neisseria* species was sequenced, making up the final three percent of the product yield.

Figure 1.

a. Staphylococcus aureus



b. Staphylococcus epidermidis



c. Streptococcus gordonii

Figure 1. Alignment graphs (5a-c) served as visual representations of each bacterial sequence. On the above horizontal axis of each graph, the chromatogram data is shown. The vertical axis shows qualitative probability of a specific nucleotide present at each segment of DNA. These 4 samples serve as representative data for the entire sample size, with 3 of these species being noted as pathogens of interest (Figures 5a, 5b, and 5c).

Overall, samples showed superior growth over the four day incubation period at 37℃, with several 30℃ plates also performing considerably well during this period of time. Additionally, there were several species whose performance was independent of a nutrient enriched media, while others showed better performance in one media type.

Table 1.

Species	***Media***	***Temperature (℃)***	***Region of Swab***	***Frequency***
Streptococcus gordonii^	TSB	37℃	GMS	1
Staphylococcus epidermidis^*	TSB-CS	30℃ and 37℃	GMS	4
Staphylococcus aureus^*	TSB-S,CS	37℃	GMS/AFS/TMBS	12
*Rothia dentocariosa**	TSB-S,CS	37℃	GMS	4
*Bacillus cereus**	TSB-C,S,CS	37℃	AFS/TMBS	6

Table 1. (Abbreviated list) Successfully identified bacterial specimens were logged with consideration of various independent variables that may have contributed to growth of a specimen. Tryptic Soy Broth (TSB), cracker media (C), sugar media (S), and cracker + sugar media (CS) were varied media that each sample experienced following the initial round of sequencing. Species that were identified during the initial round of sequencing are noted with a caret (^). The first three rounds of sequencing used only general mouth swabs (GMS), and the fourth round used anterior facial surfaces (AFS) and third molar buccal surfaces (TMBS). If a single species was identified under varying conditions, it was noted with an asterisk (*), and each condition was logged in the appropriate column.

Table 2.

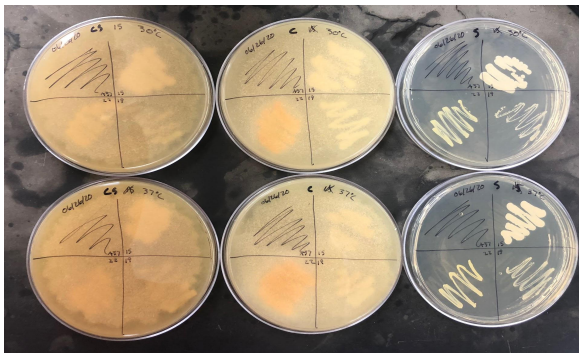
Species	***Pathogenicity***	***Virulence***	***Associated Diseases***	***Link to Periodontal Disease***
Streptococcus gordonii	Pathogenic-Opportunistic	Low	Pneumonia, Endocarditis, Meningitis	Colonizer of biofilm
Staphylococcus epidermidis	Pathogenic	High	Pneumonia, Endocarditis, Meningitis	Present in the subgingival environment
Staphylococcus aureus	Pathogenic	High	Pneumonia, Endocarditis, Meningitis	Adheres to biofilm, pathogenic through bacteremia

Table 2. (Abbreviated list) All identified species were classified on various factors that are associated with pathogenic bacterium. For the purpose of this experiment, virulence was defined in terms of effectiveness when opportunistic. Opportunism refers to the ability of causing disease in an immunocompromised individual. Information used to fill out the table came from citations: 11, 12, 15, 18, 19, 20.

Streptococcus gordonii, Staphylococcus aureus, and *Staphylococcus epidermidis* were all noted as pathogens of high interest during side experimentation due to their unique roles in the contribution to periodontal disease progression. The species were isolated onto nutrient enriched media, incubated at 37℃ and 30℃, and each species' performance was qualitatively measured after four days of incubation.

Figure 2.

a.



b.

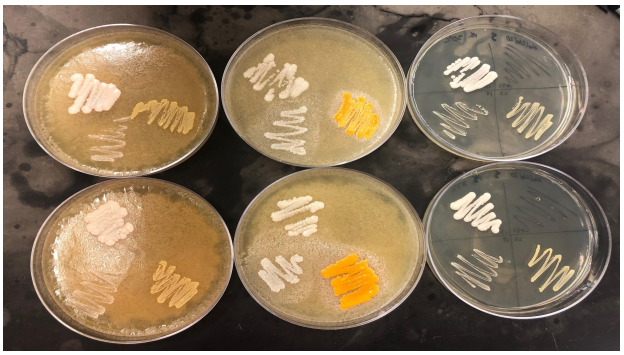


Figure 2. Pathogens of interest isolated onto nutrient enriched media (Figures 2a and 2b). In each figure, the plates are arranged by media components, as well as temperature. The arrangement (from left to right) is cracker and sugar media, cracker media, and sugar media. Likewise (from top to bottom) are 30℃ and 37℃ incubation temperatures. Figure 2a quadrants are representative as follows (clockwise from top right): Streptococcus gordonii, Staphylococcus epidermidis, and Staphylococcus aureus. Figure 2b shows a horizontally flipped arrangement of Figure 2a, and corresponds counterclockwise from top left.

Antibiotic and hygiene testing was conducted following the characterization of various species. Four different antibiotics were used to test each species' resistivity. Equate™ Antiseptic Oral Rinse was used as a form of hygiene on each sample. All measurements were made qualitatively by examining ring size around antibiotic tabloids and opacity of bacterial growth across the plate when exposed to a form of hygiene.

Table 3.

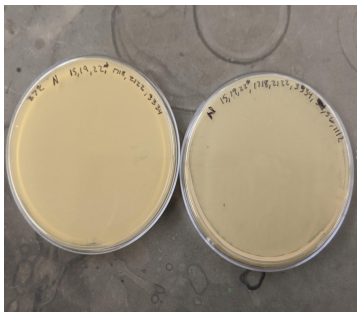
Species	***Penicillin***	***Streptomycin***	***Chloramphenicol***	***Tetracycline***	***Antiseptic oral rinse***
Streptococcus gordonii	X	O*	X	X	O
Staphylococcus epidermidis	O	X	X	X	O
Staphylococcus aureus	O*	O*	X	X	O

Table 3. Three highly virulent pathogens were examined under various antibiotic and hygiene specific tests.. "X" indicates susceptible, "O" indicatates resistive. Asterisks (*) were used to note that there were signs of susceptibility, but antibiotics possibly turned ineffective overtime.

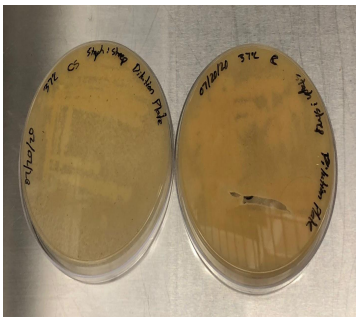
Streptococci and *Staphylococci* species were consolidated on two TSB plates in a lawn, and incubated at 30℃ and 37℃. This was a competitive growth measure that would allow for an analysis of potential synergistic relationships present between each species on a macroscopic scale.

Figure 3.

a.



b.



c.

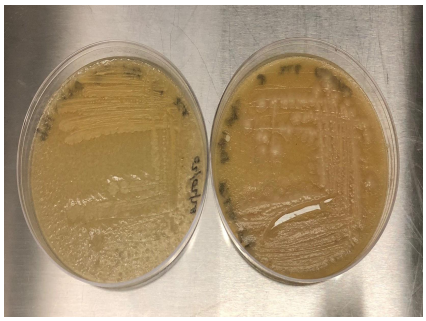


Figure 3. Staphylococcus sp. And Streptococcus sp. (left) and all species (right) that were sequenced over the duration of experimentation were consolidated onto neutral media plates (Figure 8a). Staphylococcus sp. and Streptococcus sp. were quadrant streaked onto nutrient enriched media (Figures 3b and 3c).

Discussion

The choice to incubate each sample at these two temperatures was not arbitrary: 37℃ was chosen because the average temperature in the human body is 37℃, whereas 30℃ was the average room temperature in the lab. Temperature preference was not an independent variable that changed intrinsic properties of bacterial species. The use of two different temperatures, 37℃ and 30℃, helped show how quick a particular species would grow under temperature conditions of normal body temperature and room temperature. When examining all thirty-eight successfully sequenced samples, it was determined that nearly all samples performed better when incubated at 37℃ than 30℃. This was a result that was expected because when studying the oral ecosystem, it was hypothesized that if there were to be samples that were successfully sequenced, they should be able to perform well under temperature conditions that were close to identical to that of the human body.

There was a significant amount of focus placed on *Streptococcus gordonii, Staphylococcus aureus,* and *Staphylococcus epidermidis*. This was because these species were identified early on in the experiment, which allowed more time for experimentation, but also due to each species ability to play roles in the formation of oral biofilm. Several characteristics were examined to evaluate the performance of each species, particularly colorization and intensity of color. From a qualitative stance, oral biofilm can be easily identified as plaque. The choice to focus on *Streptococcus gordonii* was solely due to its ability to form plaque [6]. This biofilm can have a colorless or pale yellow hue that is distinct when sighted, and was something that was taken note of when observing each species, especially *Streptococcus gordonii*. When examining this characteristic of *Streptococcus gordonii* on nutrient enriched media (Figure 2), this was as expected, and was not dependent upon media type nor temperature of incubation. It could be stated that there was a slight increase in the intensity of coloration for each species as the amount of nutrients changed from TSB to TSB-S to TSB-C to TSB-CS (in ascending order from left to right).

The antibiotic resistance testing results were as expected, however, the hygiene susceptibility testing was not. There appeared to be no effect from the application of antiseptic oral rinse on the bacterial samples. It can be assumed that the presence of discontinuity in the opacity of bacterial growth resulted from the re-arrangement of particles during the application of the mouthwash and not from the components of the mouthwash itself. As this was a surprise at first, more information was discovered and collected about the true effects of over-the-counter antiseptic oral rinse. In an article published by the American Dental Association (ADA), which was last revised in August of 2019, studies showed firm evidence that the sole application of any form of over-the-counter

antiseptic oral rinse will provide negligible protection against periodontal disease. Additionally, it was explicitly stated that these forms of antiseptic oral rinse is not an adequate substitute for other, more effective, forms of oral hygiene (i.e. brushing and flossing) [7]. With this information to consider, the hygiene susceptibility testing proved the notion that the sole application of over-the-counter antiseptic oral rinse is not an adequate practice of oral hygiene, and will not deter the growth of various pathogens that play pivotal roles in the formation and progression of periodontal disease. Alternative, more effective, options that could show effective results against periodontal disease would be prescribed antiseptic oral rinse from any practicing dental surgeon. For comparative purposes, the method tested in the experiment would be the equivalent for prescribing anti-inflammatory medicine to combat the effects of open-heart surgery.

The consolidation of the pathogens of interest onto one plate of TSB and TSB-C nutrient was an ecological study to examine the interaction between the various species (Figure 3). It was hypothesized that the *Streptococci* bacteria would outperform *Staphylococci* because of the roles each play in the formation of oral biofilm. Unfortunately, it could not be determined which genus performed best, and would only be interpretable if Sanger sequencing were used on growth sites of interest, which was the same procedure used throughout the entire experiment. In future experimentation, it would be ideal to conduct a more in depth analysis of the interactions between species of these two genuses and gather more information on what growing conditions are optimal for the biofilm.

The frequency of sequenced species was decided to be recorded for several reasons (Table 1). In this experiment, the sample size was less than expected due to the COVID-19 pandemic. This hindered the ability to expand the sample size across multiple demographics. From the six subjects who provided saliva samples, there were several species that were present across multiple subjects, while others were only present once. By recording frequencies, potential outliers could be noted as the experiment progressed. Species whose frequency was relatively high, such as *Staphylococcus aureus, Bacillus cereus, Rothia dentocariosa,* and *Staphylococcus epidermidis,* could suggest these species could potentially be found across multiple demographics, and are not outliers in the experiment. No species could be considered outliers from the data collected thus far, however, as experimentation continues in the future, these data can be referred to as a guided reference of what to expect.

Cardiovascular Diseases Potentially Linked with Periodontal Disease

Many of the identified bacteria in (Table 2) have been associated with endocarditis. Endocarditis is a type of cardiovascular disease where the inner lining of your heart chambers and valves becomes infected [8]. The most common cause of endocarditis is bacterial infection commonly caused by *Staphylococcus* and *Streptococcus* species[9]. Infective endocarditis also known as bacterial endocarditis is caused by bacteria that enters the bloodstream [10]. Bacterial species can enter the bloodstream during dental procedures that is why antibiotics are often given before the procedure to reduce the risk of bacterial infection. It should also be noted that when one has periodontal disease the gums can often swell or bleed allowing periodontal bacteria to enter the bloodstream. The evidence that *Staphylococcus* and *Streptococcus* species can cause endocarditis without any underlying conditions is strong. *Streptococcus gordonii* has been reported to cause infective endocarditis in an immunocompetent 31 year old male following a dental procedure that resulted in a dental abscess [11]. *Staphylococcus aureus* is the most common pathogen to cause infective endocarditis around the world, even affecting patients who are immunocompetent with no underlying conditions [12]. However, many of these infections seem to occur after surgery. It must also be noted that *Staphylococcus aureus* has become methicillin-resistant known as MRSA which has caused rates of infection to increase significantly [12]. *Rothia dentocariosa* causes

endocarditis infections however having endocarditis secondary to *Rothia dentocariosa* is rare [9]. Of the 20 cases of infective endocarditis secondary to *Rothia dentocariosa* 18 had predisposed heart conditions while the other 2 had poor dental hygiene or procedures predisposing them to *Rothia dentocariosa* infections therefore causing endocarditis[9].

There is reason to believe that there is a possible link to cardiovascular disease and periodontal disease via endocarditis. If *Staphylococcus* and *Streptococcus* species get into the bloodstream there is a high risk of that periodontal bacteria solely causing endocarditis even if the patient has no underlying conditions and is immunocompetent. Likewise, it could be believed *Rothia dentocariosa* has a much lower risk of causing endocarditis as the patient would need to have underlying heart conditions or be immunocompromised for there to be a risk of developing endocarditis. As a result we believe this is strong evidence of periodontal bacteria causing endocarditis a type of cardiovascular disease.

Neurodegenerative Diseases Potentially Linked with Periodontal Disease

A large majority of the bacteria identified in the experiment was associated with causing different types of bacterial meningitis (Table 2). Meningitis causes the meninges surrounding the brain and spinal cord to swell usually causing migraine like headaches [13]. Bacterial meningitis is caused by bacteria and is very serious as it can cause death in as little as a few hours [14]. Most have the ability to recover, however, meningitis can cause permanent disabilities such as brain damage, hearing loss, and learning disabilities [14]. Bacterial meningitis can be easily spread through close contact with an infected person, such as kissing, or even inhaling bacteria present in air because of a cough or sneeze[14].

One of the bacterial species identified that can cause meningitis was of the *Neisseria* genus. The genus *Neisseria* contains two important human pathogens *N. Meningitidis* which causes meningococcal meningitidis and *N. gonorrhoeae* which causes gonorrhea [15]. *N. gonorrhoeae* infection occurs often but has a low mortality rate[15]. *N. Meningitidis,* which causes meningococcal meningitis, is rare but has a high mortality rate[15].

Staphylococcal meningitis is a type of bacterial meningitis characterized when a *Staphylococcus* bacterial species is found in the body or cerebrospinal fluid [16]. This type of meningitis is either hospital or community acquired and, although it is rare, it is very deadly as *Staphylococcus* bacteria are often resistant to medication [16]. When *S. aureus* or *S. epidermidis* bacteria are found, it can be assumed that the meningitis was caused from a surgical procedure [16]. The long term effects of Staphylococcal meningitis, if survived, include: brain damage, fluid build up between brain and skull which can cause the brain to swell, hearing loss, seizures, and staph infection in other areas of the body [17].

There were also a number of bacterial species in Table 2 that have been reported causing bacterial meningitis, but these were often rare cases. *Bacillus subtilis* has shown links to bacterial meningitis however this is often a rarity. A case report detailed one's development of pyogenic meningitis which led to a Central Nervous System infection when an immunocompetent male had very poor hygiene on top of severe tooth decay, resulting from periodontitis [18]. *Rothia mucilaginosa* has also been linked to causing bacterial meningitis, which has shown to be fatal. In one case report, an immunocompromised woman developed bacterial meningitis from *R. mucilaginosa,* which was resistant to all medicine and antibiotics. As a result, the woman would die because of refractory intracranial hypertension [19]. *Streptococcus oralis* has also been reported in causing a rare case of bacterial meningitis. It was found that *S. oralis* can cause meningitis when the dental cavity is manipulated during surgery, but the patient must also have decreased immune function for the bacterial meningitis to develop [20].

Therefore it can be suggested that there is a possible link to neurodegeneration and

periodontal disease via meningitis. As you can see in the above discussion many periodontal bacteria have been found to cause different types of bacterial meningitis. Some forms of meningitis, such as meningococcal meningitis and staphylococcal meningitis, are very common and contagious. There are other, more rare, forms of bacterial meningitis are caused by certain types of bacteria such as *Rothia mucilaginosa, Bacillus subtilis, and Streptococcus oralis.* However, one thing that is common are the negative effects of bacterial meningitis if survived. The long-term consequences could show brain damage, hearing loss, seizures, and disability; all of which are signs of neurodegeneration [21].

Conclusion

Based upon the bacterial species that were collected and successfully sequenced throughout the ten-weeks of research and experimentation, it can be suggested that there may be pathogens that are associated with cardiovascular disease and neurodegeneration that could also be linked with periodontal disease.

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