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Biofilm mediated antibiotic resistant oral bacteria among Parkinson's patients

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Abstract

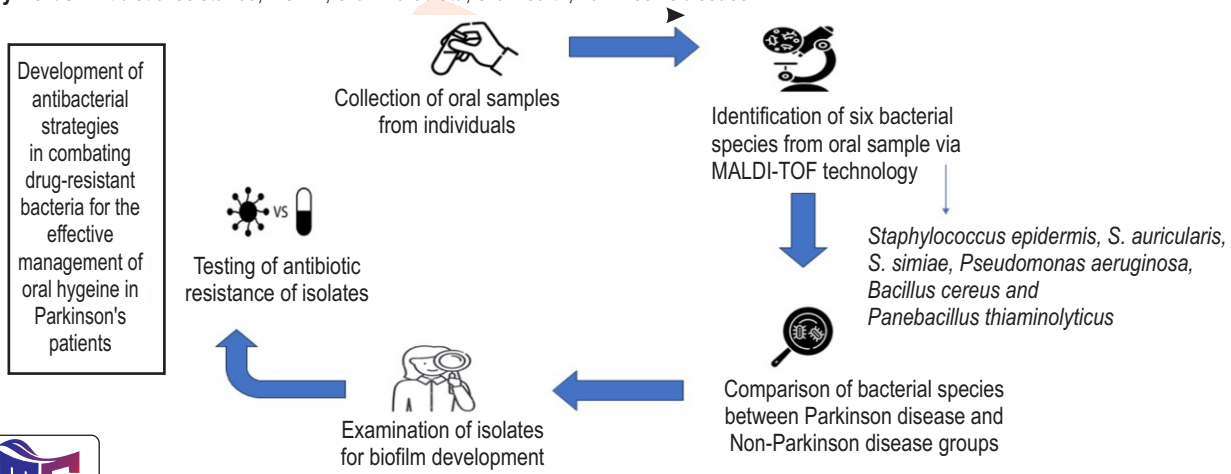
Aim: The aim of this study was to examine the oral microbe biofilm and antibiotic resistance in individuals with Parkinson's disease compared to those without the condition.

Methodology: In this study, the oral bacteria of older patients with Parkinson's disease and those without the condition were examined for antibiotic resistance and biofilm formation. In this study, the microbiologists examined oral samples collected from 33 individual, out of which 18 sufferred from Parkinson's disease while the remaining 15 individuals were normal. This case involved the use of the Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) technology to identify six different species.

Results: The analysis of oral samples showed the presence of six isolates viz. *Staphylococcus epidermis, S. auricularis, S. simiae, Panebacillus thiaminolyticus, P. aeruginosa* and *Bacillus cereus.* Out of these, *P. thiaminolyticus* was absent in the control group. The incidence of oral microorganisms was somewhat higher in Parkinson's disease patients than control individuals, however, there was no discernible variation in the oral bacterial strains found in the two research groups. The isolates underwent further analysis following Congo Red Agar and Tissue Culture Plate methods and showed positive result for biofilm formation. Each isolate found in the Parkinson's disease groups were found resistant to at least five antibiotics used.

Interpretation: The ability to produce biofilm was present in approximately 83.3% of the isolates from the Parkinson's disease group and 80% of those from the control group. This study provides a deeper understanding of the relationship between biofilm-producing, antibiotic-resistant oral bacteria and Parkinson's disease, and also address the need for effective management of oral hygiene in Parkinson's patients.

Key words: Antibiotic resistance, Biofilm, Oral microbiota, Oral health, Parkinson's disease



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Introduction

Parkinson disease is a age-related motor illnesses, prevalent with advancing age in people (Van Den Eeden *et al.,* 2003). People suffering from Parkinson disease show varied symptoms, both motor and non-motor with Tremor, Bradykinesia, and stiffness being the most common motor symptoms (Jiménez and Vinger, 2012), which may obstruct automated minor hand motions (Schwarz *et al.,* 2006), including the capacity to brush teeth leading to oral health deterioration (Müller *et al.,* 2011). These symptoms have been linked to enteric nervous system neuropathology, which is associated with the brain protein alpha-synuclein. Identical changes have been observed in the upper oesophagus, larynx and oropharynx mucosal sensory nerve terminals. Parkinson's disease often manifests after the age of 40 and primarily affects males (Mu *et al.,* 2017). Because of extrinsic variables like germs in the upper respiratory tract and gastrointestinal tract, olfactory and gastrointestinal tissues may get damaged due to sickness (Fansano *et al.,* 2015). This may initiate rapid degeneration of nerve cells, leading to Parkinson's disease (Lema Tomé *et al.,* 2013). Several studies have been undertaken that effectively link the gut microbiota's function in Parkinson's disease (Scheperjans *et al.,* 2015; Nielsen *et al.,* 2021).

Periodontal disease, which has been connected to Parkinson's disease (Müller *et al.,* 2011; Kamer *et al.,* 2020) has a significant influence on the latter. Numerous studies have also suggested that oral microbiota may have a role in several human illnesses, including diabetes and cardiovascular diseases (Kumar, 2017; Giordano *et al.,* 2022). Patients with Parkinson's disease who have a disturbed oropharyngeal status may also see repeatable changes in the salivary microbiota that set them apart from normal. Chewing/ biting issues, loss of taste, tooth movement, and xerostomia are all significant symptoms of Parkinson's disease (Van Stiphout *et al.,* 2018). Oral cavity issues are one of the expanding non-motor signs and symptoms of Parkinsonism (Simonet *et al.,* 2022). Due to dental and periodontal conditions, it might be challenging for Parkinson's disease patients to maintain good oral hygiene. There are several reasons why patients suffering from Parkinson's disease cannot maintain their dental hygiene. These include, motor impairment (reduced dexterity of the arms and fingers may make it difficult to do the necessary daily oral hygiene treatment), apathy, depression and dementia (Hanaoka and Kashihara, 2009; Vanbellingen *et al.,* 2010; Debowes *et al.,* 2013).

Previous studies have reported deteriorated periodontal health, more missing teeth, caries, and dental biofilm in patients suffering from Parkinson's disease as compared to healthy individuals (Petersen, 2003; Einarsdóttir *et al.,* 2009; Muller *et al.,* 2011). The microbes that form biofilms for defense are more resistant to various classes of drugs (Rather *et al.,* 2021). The advantage of bacterial biofilms to an organism's survival processes can be related to virulence, pathogenicity or antibiotic resistance (Brandwein *et al.,* 2016; Vestby *et al.,* 2020). Aspiration pneumonia stands out as a prominent cause of mortality in Parkinson's disease, with an elevated risk attributed to oral dysbiosis resulting from inadequate oral hygiene (Rozas *et al.,* 2021). In view of the above, the aim of this study was to examine and compare the oral microbe biofilm and antibiotic resistance in Parkinson's disease patients with normal indviduals.

Materials and Methods

Study subjects and sampling: Each participant in this research received questionnaire comprising information about their demographics, age, gender, symptoms, duration of disease and dental hygiene. In total thirty-three (No=33) saliva samples from the oral cavity were collected, 15 from Parkinson's disease patients (three were removed), and 15 from normal participants. Since the sampling was non-invasive, all human volunteers and dental professionals gave written consent before sample collection. The individuals in the Parkinson's disease group met the standard diagnostic criteria for late-onset Parkinson's disease, which include bradykinesia, stiffness, or a resting tremor, and had previously been diagnosed by a neurologist (Schwarz *et al.*, 2006; Postuma *et al.,* 2015). Participants with periodontal disease were not excluded from the study, nor were those with any known conditions affecting the mouth, salivary glands, nose, throat or ears. Additionally, individuals who had taken antibiotics within a month prior to the sample collection date were also included. There was no thorough clinical assessment of the subjects' oral cavity or dental health. Saliva samples were obtained by gently stroking the buccal and sublingual mucosa both sides of the mouth using a sterile cotton swabs. Samples from this region were taken into consideration because of their close proximity to the parotid and submandibular ducts. Cotton swabs containing samples were sealed in sterile vials and delivered straight to the laboratory under ice-cold conditions within 48 hr of sample collection (Byrne *et al.,* 2022).

Identification of bacteria using MALDI-TOF Mass Spectroscopy: MALDI-TOF technique was used to examine each isolate at the Microbiological Laboratory Pvt. Ltd., Coimbatore, India. The samples were inoculated at 37°C onto nutrient agar for 24 hr. A microtiter plate was used to hold each culture. A lysis solution was then added to the bacterial sediment, therefore one litre of. aliquot of the matrix solution—containing 2.5% trifluoroacetic acid and 50% acetonitrile diluted with alpha-ciano-4-hydroxy-cinamic acid from Sigma-Aldrich®—was added. Nitrogen laser (337 nm) was used in the mass spectrometer to create a spectra for each sample, following a straight path. The standard configuration for bacterial identification was employed to assess the spectra in the mass range of 2000 to 20,000 m sec^{-1} using the MALDI Biotyper 2.0 application. Later the sample spectra were contrasted with the database references.

Screening for microorganism producing biofilm

Congo Red Agar method: Oral isolates were streaked on Brain Heart Infusion agar, which consisted of 5% sucrose and 0.08%

Congo Red dye. The plates were incubated for 24 hr at 37 °C (Bose *et al.,* 2009). The isolates producing biofilms showed black colonies whereas the non-biofilm producing isolates developed red colonies.

Tissue culture plate method: An overnight culture in nutrient broth and a 1:100 dilution in BHI broth were used for the biofilm development experiment in microtiter wells. Using a flat-bottom 96-well polystyrene microtiter plate, a 200µl of cell suspension was added to each well. Following a 48 hrs incubation period at 35˚C, the detached cells underwent three gentle rinses with sterile distilled water. Subsequently, the bacteria adhered to the surface were stained using crystal violet, recleaned and de-stained with ethanol-acetone (95:5, v/v). Optical density was read at 570 nm wavelength (OD) 570nm using 200µl of the combined solution that was transferred to a 96-well microtiter plate (Pierce *et al.,* 2008). Micro-ELISA Auto-reader recorded the absorbance. Each test was run thrice, and the mean OD_{570} value of the wells that were tested was used. Based on the ODs in the BHI broth, the biofilm formation was separated into three categories: biofilm non-formers (OD570<0.2), biofilm formers of weak level (OD570 in the range of 0.2-1.0), and biofilm formers of strong level (OD570 >1.0) (Bose *et al.,* 2009).

Antibiotic sensitivity test: The bacteria screened for biofilm production were further analysed for antibiotic susceptibility test. A sterile cotton swab was dipped into the overnight culture, employing the subsequent drugs: Imipenem (10 mcg), Linezolid (30 mcg), Methicillin (5 mcg), Minocycline (30 mcg), Ampicillin (10 mcg), Piperacillin-Tazobactam (100/10 mcg), Tigecycline (15 mcg), Cefepime (30 mcg), Cefotaxime (30 mcg), Ceftazidime (30 mcg), Co- Trimoxazole (25 mcg) and Ceftriaxone (30mcg) (Hi-Media, Mumbai). The Mueller-Hinton agar medium (Hi-Media, Mumbai) was thoroughly streaked with swabs, and the antibiotic disc was gently placed above it with the help of tweezers. The plates were incubated at 37 °C for 24 hr. The inhibition zones on the plates were checked after the incubation period. The zones were measured and interpreted in accordance with the Clinical and Laboratory Standard Institute's Guidelines (Javiya *et al.,* 2008; CLSI, 2016).

Statistical analysis: All experiments were performed in triplicate and the data were analyzed using a Student's t-test. Statistical significance was determined at a p-value threshold of 0.05.

Results and Discussion

In total thirty-three (N=33) participants including 15 Parkinson's disease patients and 15 normal individuals (and 3 excluded) participated in this study. Participants suffering from Parkinson's disease were 50 years old. The prevalence of Parkinson's disease increases with advancing age, with a notable rise observed in individuals aged 60 to 64 (33.33%) and those aged 65 and above (50%), according to the distribution among Parkinson's disease patients in the specified age groups. It has been found that illness incidence advances with age and that men are more likely to suffer from Parkinson's disease than women. Additionally, it was reported that each patient suffering from Parkinson's disease experienced resting tremors.

The proportion of Parkinson's disease cases presenting various symptoms within the study group was analyzed, revealing tremor as the most prevalent symptom, affecting all individuals (100%). Additionally, difficulty in walking (66.67%) and excessive salivation (38.89%) were also commonly reported, while memory loss (27.78%) and depression (11.11%) were less frequently observed among the affected individuals. Salivary microbes from Parkinson's disease patients and healthy individuals were compared (Table 1) showing *S. epidermidis* to be present in 16.67% of PD individuals compared to 13.34% in the control group. *S. auricularis* was found in 5.56% of Parkinson's patients, significantly lower than the 20.0% observed in the control group. Notably, *P. thiaminolyticus* was detected in 33.33% of individuals with Parkinson's disease but was absent in the control group.

B. cereus was present in 11.11% of Parkinson's patients, whereas it was more prevalent in the control group (33.34%). Finally, *P. aeruginosa* was present in 22.22% of Parkinson's patients and 26.67% of controls. These variations suggest that Parkinson's disease may influence the composition of the oral microbiota, potentially impacting oral health and the propensity for certain infections. Saliva samples from both group showed the presence of *Staphylococcus species* viz., *S. epidermidis, S. auricularis*, and *S. simiae*, The prevalence of microorganisms across the two groups did not differ significantly, with the exception of *P. thiaminolyticus,* which was absent in control participants. *P. thiaminolyticus* predominated (33.33%) in the saliva of Parkinson's disease patients, but *B. cereus* was found in higher percentage (33.34%) of all other isolates in the saliva of normal patients. The presence of *P. aeruginosa* was similar in both patients. The identified oral bacterial species were tested for the existence of biofilms by Congo red agar plate method. The oral isolates showed dry, black colonies that suggested the formation of biofilms, while red colonies revealed the presence of non-biofilm producers (Table 2).

From the tissue culture plate analysis, a high prevalence of biofilm was observed among oral isolates obtained from individuals with Parkinson's disease, with 61.29% of samples

Table 1: Microbiota profiles in the oral cavity of individuals with and without Parkinson's disease

Microorganisms	Parkinson's disease (%)	Control (%)
S. epidermidis	3 (16.67)	2 (13.34)
S. auricularis	1 (5.56)	3 (20.0)
S. simiae	2 (11.11)	1 (6.67)
P. thiaminolyticus	6 (33.33)	0
B. cereus	2 (11.11)	5 (33.34)
P. aeruginosa	4 (22.22)	4 (26.67)

Table 2: Prevalence of biofilm development among oral microflora in Parkinson's disease and control populations

	Parkinson's disease		Control	
Microorganisms	**Biofilm** Producing (%)	**Non-biofilm** Producing (%)	**Biofilm** Producing (%)	**Non-biofilm** Producing (%)
S. epidermidis	2 (66.67)	1 (33.33)	1 (50)	1 (50)
S. auricularis	1 (100)	0	2 (66.7)	1 (33.33)
S. simiae	1 (50)	1 (50)	1 (100)	0
P. thiaminolyticus	0	6 (100)	-	-
B. cereus	2 (100)	0	3 (60)	2 (40)
P. aeruginosa	3 (75)	1 (25)	4 (100)	0

Table 3: Biofilm-producing microorganism identification in Parkinson's disease and Non-Parkinson's disease Individuals

Microorganisms	Parkinson's disease	Non-Parkinson's disease
S. epidermidis	+	+
S. auricularis	++	++
S. simiae	++	+
P. thiaminolyticus	-	-
B. cereus	++	+
P. aeruginosa	++	++

Note: (+++) Strong, (++) Moderate, (+) Weak, (-) No biofilm production

showing biofilm production. The optical density (OD) measurements of stained adherent bacteria was analyzed with a micro-ELISA auto reader operating at 570 nm. Based on OD values, isolates were categorized as strong, moderate, weak, or non-biofilm producers. *Staphylococcus species* (*S. epidermidis, S. auricularis* and *S. simiae*) and *B. cereus* showed varying degrees of biofilm production in both groups, with generally higher levels observed in Parkinson's disease patients compared to controls as shown in table 3. Notably, *P. aeruginosa* demonstrated significant biofilm production in both groups. Notably, all oral isolates exhibited resistance to a minimum of five antibiotics, as shown in Table 4. Table 5 presents the relationship between antibiotic resistance patterns and biofilm-forming oral microorganisms. Each microorganism displayed resistance to multiple antibiotics, with *B. cereus* resistant to 7 antibiotics and *P. aeruginosa* resistant to 6 antibiotics, respectively (table 4). *Staphylococcus* species, including *S. auricularis, S. epidermidis,* and *S. simiae,* exhibited resistance to five antibiotics each. This suggests a concerning trend of multidrug resistance among biofilm-forming oral microorganisms associated with Parkinson's disease, emphasizing the need for effective antimicrobial strategies in managing oral health in patients.

Approximately, 0.02% people above 65 years of age all over the world suffer from Parkinson's disease caused by substantia nigra of the brain where nerve cells slowly die off. About 88.8% of the Parkinson's disease patients in this research were males, while the remaining 11.2% were females. According to Moisan *et al.* (2016) and Roohani *et al.* (2013), men are more likely to suffer from Parkinson's disease almost 1.6 times more than women. Moisan *et al.* (2016) found that the male-to-female ratio for the disease increases with age, which is in line with the results of the current investigation. Rozas *et al.* (2021), investigated the factors influencing the oral microbiota in individuals with Parkinson's disease. The findings revealed an increased presence of opportunistic oral pathogens among the patients. The study identified specific factors, such as dysphagia and drooling (both with a significance level of $p < 0.05$), along with salivary pH ($p < 0.005$), that had a significant impact on the beta-diversity of soft tissues and composition of oral microbiota. Previous research has demonstrated that non-oral species detected in the oral microbiota may function as a repository for medically significant pathogens, rather than merely being the result of contamination during sample collection (Botero *et al.,* 2012; Souto and Colombo, 2008; Fritschi *et al.,* 2008; Gonçalves *et al.,* 2009; Wang *et al.,* 2023). However, it was observed that all the six isolates were sensitive to Imipenem and Piperacillin-tazobactam antibiotics.

Staphylococcus species such as *S. auricularis, S. epidermidis* and *S. simiae* have been found to inhabit the oral cavity, and under certain conditions, it can lead to localized or systemic infections (Kloos and Bannerman, 1994). For instance, *S. auricularis,* typically found in the ear, can cause infections if it accesses sterile body sites; *S. epidermidis,* a common skin commensal, can form biofilms on medical devices and contribute to infections, particularly in immunocompromised individuals; and *S. simiae*, although less commonly discussed, can cause infections if it enters the bloodstream or other sterile areas. The frequency of *Staphylococcus* species in oral samples, as reported by Simes-Silva *et al.* (2018) and Smith *et al.* (2011), supports their common presence in the oral cavity. Their persistence can be attributed to mechanisms such as biofilm formation, which protects them from host immune responses and antibiotics (Beatriz *et al*., 1999; Bose *et al.*, 2009); adhesion to epithelial cells facilitated by surface proteins; and the development of antibiotic resistance, particularly in species like *S. epidermidis.*

Coagulase Negative Staphylococci (CoNS), especially *S. epidermidis,* pose a significant threat due to their ability to develop antibiotic resistance and form biofilms. These bacteria can adhere to oral surfaces, contributing to periodontal disease and complicating treatment due to their resistance to common

Table 4: Antimicrobial pattern for oral microorganisms isolated from Parkinson's disease subjects

Antibiotics	*S. epidermidis*	*S. auricularis*	*S. simiae*	*P. thiaminolyticus*	*P. aeruginosa*	*B. cereus*
AMP	R	R	R	R	R	R
CAZ	R	R	R	R	S	R
MET	R	R	R	R	R	R
CTX	R	R	R	I	I	R
CPM	R	R	R	R	S	R
CTR	I	R	R	R	S	R
TGC	S	S	S	S	R	S
COT	I	I	I	I	R	R
LZ	S	S	R	S	R	S
MI	S	I	I	S	R	I
PIT	S	S	S	S	S	S
IPM	S	S	S	S	S	S

*AMP- Ampicillin; CAZ- Ceftazidime; MET- Methicillin; CTX- Cefotaxime; CPM- Cefepime; CTR- Ceftriaxone; COT- Co-Trimoxazole; TGC- Tigecycline; LZ- Linezolid; MI- Minocycline; IMP- Imipenem; PIT- Piperacillin-tazobactam. **R- resistant; I- Intermediate; S- Sensitive

antibiotics like β-lactams, gentamicin and erythromycin (Asante *et al.,* 2020). These mechanisms highlight the adaptability of *Staphylococci* to the oral environment and their potential role in oral and systemic infections, underscoring the importance of monitoring and understanding their behavior to manage and prevent infections effectively. Therefore, due to the presence of CoNS in the oral cavity, Parkinson's patients may develop periodontal diseases (Manandhar *et al.,* 2021). *S. auricularis* has hardly ever been found in oral infectious diseases (Braga *et al.,* 2005). Ouyang *et al.* (2024) reported the presence of *P. thiaminolyticus* in an 80-year-old Parkinson's disease patient, which is in line with the present findings. This peculiar spore-forming bacteria, like *Paenibacillus* sp. strain VT-400, which was identified in the saliva of patients with acute lymphoblastic leukaemia, has not been reported in humans (Tetz *et al.,* 2015). *P. thiaminolyticus* in oral samples of Parkinson's patients may be the first of its kind. Furthermore, before Paenibacillus species were identified as human pathogens, they were known to cause respiratory and urinary tract infections (Kim *et al.,* 2010; Padhi *et al.,* 2013).

Patients with periodontitis reported a notably higher prevalence of *P. aeruginosa* in their saliva and subgingival biofilm samples, according to Souto *et al.* (2014). The bacterium was found in both the participants in this study, indicating their role in the etiopathogenesis of periodontal diseases in Parkinson's patients. Bakke *et al.* (2011) found that dental plaque, food particles, and periodontal health are more evident in patients with advanced Parkinson's disease (Hansen *et al.* 1993). Food debris may be the cause of *B. cereus* colonization in the oral cavity, which may be overlooked at early stages of pulmonary and systemic infections in immunocompromised individuals' development (El Saleeby *et al.,* 2004). The salivary flow rate is essential for maintaining optimal oral health in Parkinson's patients because it plays a preventative function against plaque and germs from mucosal and dental surfaces (Kennedy *et al.,* 1994; Müller *et al.,* 2011). Previous studies have reported that

Table 5: Relationship between the pattern of antibiotic resistance and the biofilm-forming oral microorganisms

Microorganisms	Parkinson's disease
B. cereus	AMP, CAZ, MET, CTX, CPM, CTR, COT (7)
P. aeruginosa	AMP, MET, TGC, COT, LZ, MI (6)
S. auricularis	AMP, CAZ, MET, CTX, CPM, CTR (6)
S. epidermidis	AMP, CAZ, MET, CTX, CPM (5)
S. simiae	AMP, CAZ, MET, CTX, CPM (5)

*AMP- Ampicillin; CAZ- Ceftazidime; MET- Methicillin; CTX- Cefotaxime; CPM- Cefepime; CTR- Ceftriaxone; COT- Co-Trimoxazole; TGC- Tigecycline; LZ- Linezolid; MI- Minocycline

saliva includes proteins or glycoproteins that aid in the formation of enamel layer, bacteria to cling to surfaces to form plaque (Holt *et al.,* 1988; Zimmerman *et al.,* 2013; Fiorillo, 2019; Hanisch *et al.,* 2019). Microbes producing biofilms have caused numerous problems in agriculture, medicine and daily living (Shineman *et al.,* 2014). Germs that form biofilms are also assumed to be resistant to antibiotics due in part to their slower rate of cell proliferation when compared to free-living planktonic cells (Chuard *et al.,* 1993). *P. aeruginosa* and S. epidermidis were shown to be poor biofilm producers in the oral bacteria of Parkinson's patients, but *B. cereus, S. auricularis,* and *S. simiae* were shown to be moderate biofilm builders (Hsueh *et al.,* 2006; Okajima *et al.,* 2006). *P. thiaminolyticus* isolated from Parkinson's disease was unique as it did not form biofilm. Santos *et al.* (2015) revealed the prevalence of *Staphylococcus* spp. in the subgingival biofilm. *B. cereus* in this study showed resistance to most of the antibiotics tested, similar to report of Owusu-Kwarteng *et al.* (2017). *S. auricularis* and *P. aeruginosa* both showed resistance to Ampicillin, Methicillin, Tigecycline, Co-Trimoxazole, Linezolid and Minocycline (Brown *et al*., 2012). The presence of spores has significantly contributed to both the persistence of infections and the spread of antibiotic resistance. Five different antibiotics were resistant to *P. thiaminolyticus* (Barra-Carrasco *et al.,* 2014). Furthermore, it was found that

among the *Staphylococcus* species isolated from oral samples, *S. auricularis* was resistant to Ampicillin, Ceftazidime, Methicillin, Cefotaxime, Cefepime and Ceftriaxone, while *S. epidermidis* and *S. simiae* were both resistant to Ampicillin, Ceftazidime, Methicillin, Cefotaxime and Cefepime. Conversely, all species of *Staphylococcus* were found to be susceptible to linezolid, aligning with the findings of Abdel Halim *et al.* (2018). This consistency underscores linezolid's effectiveness as a treatment option against *Staphylococcus* infections, highlighting its potential as a reliable antibiotic in the face of rising antibiotic resistance.

The susceptibility of *Staphylococcus* species to linezolid reinforces its role in clinical settings, particularly for infections that are resistant to other antibiotics. Most people agree that clinical isolates with higher antibiotic resistance levels arise from repeated medication exposure (Antoniadou *et al.,* 2013). Cantas *et al.* (2013) suggest that there might be a connection between the environment and the development of antibiotic resistance in microorganisms. In a similar study, Fleury *et al.* (2021), observed higher abundance of certain bacterial species, including *Streptococcus mutans*, *Kingellaoralis,* Actinomyces AFQC_s, Veillonella AFUJ_s, *Scardovia, Lactobacillaceae, Negativicutes,* and *Firmicutes* were observed in patients.

Conversely, there was a lower abundance of *Treponema* KE332528_s, *Lachnospiraceae* AM420052_s, and the phylum SR1 in the same patient group (Fleury *et al*. 2021).The heightened prevalence of multidrug-resistant CoNS among Parkinson patients, as indicated by our research, is concerning. Furthermore, this observation suggests a correlation with compromised dental hygiene and deteriorating oral health, likely attributable to deficiencies stemming from Parkinson's disease, such as impaired motor control affecting oral care practices.

Investigating oral microbiota of Parkinson's patients can help develop new treatment strategies and provide a better understanding of how the illness progresses. Furthermore, oral bacteria are important pathogenic agents of pneumonia and other opportunistic infections in people with Parkinson's disease. To completely characterize the microbial population and understand how they affect Parkinson's disease, advanced study is needed. These microorganisms can serve as a Parkinson's disease diagnostic marker. Patients with Parkinson's disease should visit their dentist regularly, and the care giver should also get dental hygiene training.

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Authors' contribution: J. Joel: Contributed to the research idea and writing of the manuscript; **P. Xavier:** Contributed to the research idea including framing of questionnaire for collecting primary data; **J.S. Pillai:** Contributed to the research article through writing; **T.S. Sujitha:** Contributed by writing the research article and conducting the research work; **S. Sree:** Collaborated in writing and editing the manuscript; **A. Iyer:** Assisted in writing and editing the manuscript; **S. Murugan:** Provided the initial research idea, assisted in coordination, and participated in the final correction of the paper.

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Ethical approval: The researchers had maintained anonymity with verbal consent from the patients under the meticulous guidance of a medical expert, who also requested anonymity. Since oral samples were collected through non-invasive procedures, no formal ethical approval was required. However, the study adhered to ethical standards, ensuring the utmost respect for the rights and privacy of the participants. The research team remained committed to upholding ethical principles and safeguarding the integrity of the study while contributing valuable insights to the understanding of Parkinson's disease.

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Data availability: Not applicable.

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