

Evaluating the use of blood pressure and oral microbiome screening in primary care dental clinics

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Abstract

Blood pressure and oral microbiome screening are gaining popularity in non-medical settings, the hypotheses communicated to hypertensive patients being that: altering the abundance of certain bacterial species via oral hygiene could be beneficial for cardiovascular health, especially nitrate reducing species that lower blood pressure. Two-hundred participants (58.4±10.8 y/o) therefore underwent Systolic (SBP) and diastolic (DBP) blood pressure screening in a primary care clinic at some other time than dental treatment. 43.5% were normotensive (NT; SBP<120mmHg; DBP<80mmHg) and 31.5% were hypertensive (HT; SBP>140mmHg; DBP>90mmHg). After confounding factors were removed (NT-OM and HT-OM), the relative abundance of total oral nitrate-reducing bacteria (ONRB; determined using 16S rRNA, sequencing) and oral nitrate reducing activity (ONRA; determined using high-performance liquid chromatography; HPLC) increased in HT-OM (N=15) compared to NT-OM (N=24) (P=0.031). In agreement, some nitrate reducing bacterial species were higher, such as *Neisseria flavescens* however, *Neisseria subflava* were lower in HT-OM (P<0.05). Using strict protocols, primary care dental clinics can therefore reliably identify undiagnosed hypertension alongside changes to the oral microbiome. However, given that functional species-level differences may not align with expected systemic changes, further research is needed before dental clinics use oral microbiome screening to identify specific bacteria for guiding oral hygiene to manage hypertension.

INTRODUCTION

Hypertension is a disease of high blood pressure, which is the main risk factor for adverse cardiovascular events, including heart attacks and strokes [1-2]. However, hypertension is a preventable disease through lifestyle changes, dietary modifications and low-cost medications [3]. Recent evidence also demonstrates that gum disease (periodontitis) is linked to increased risk of hypertension [4]. Thus, oral hygiene measures, which reduce the abundance of pathogenic bacteria causative for periodontitis (such as *Porphyromonas Gingivalis*) via toothbrushing, interdental cleaning alongside professional mechanical plaque removal (PMPR) may reduce blood pressure (BP) in people with hypertension, albeit with varying degrees of success [5-6]. Although millions of people worldwide successfully engage in various preventative approaches for managing high blood pressure, it remains a problem if people are unaware that they have hypertension (undiagnosed). Globally, the World Health Organisation (WHO) recognises that undiagnosed hypertension contributes to over 10 million deaths annually worldwide, remaining the leading modifiable risk factor for cardiovascular disease (CVD) [7-8].

In agreement with prevention being a priority for management of cardiovascular disease, BP assessment in non-traditional settings has increased [9], with 'out of office' measurements now recommended globally, leading to American Heart Association (AHA) and European Society of Hypertension (ESH) guidelines that can be implemented to ensure readings are accurate [10-12]. However, there remains resistance around the reliability of dental clinics for such tasks, because of the risk of 'white coat syndrome' artificially elevating blood pressure [13-14]. Although of note, people who experience white coat syndrome are of higher risk of CVD [15]. Thus, in addition to following guidelines, our previously published pilot study undertook BP screening appointments separate to the dental appointments and referred cases of possible hypertension to General Medical Practitioners (GPs) [16-17] to confirm diagnosis; here we report the findings in full.

Considering oral disease, systematic review evaluating metagenomic studies found that *Atopobium*, as well as *Prevotella* and *Veillonella*, which are pathogens associated with periodontal disease, can be repeatedly found in hypertensive subjects [18]. The concept follows such that; if oral microbiome screening is conducted in the dental clinic, personalised treatment plans can be made involving oral hygiene interventions and PMPR to reduce pathogenic bacteria, including during hypertension as these people be at increased risk of periodontal disease [19]. Of note however, this ongoing practice is not yet supported by national guidelines [20]. Thus, new research studies identifying how the oral microbiome changes in undiagnosed hypertension are urgently needed, before any interventional approaches are applied.

As both oral and systemic disease are associated with oral microbiome dysbiosis [21-22], our research group has an interest in the concept of 'balancing' or diversifying the oral microbiome to restore 'health' [23]. Arguably, oral nitrate reducing bacterial species (ONRB), such as *Prevotella*, *Rothia*, *Neisseria* and *Veillonella* promote oral and systemic 'health', as they reduce nitrate from endogenous or exogenous (diet) sources to nitrite [24]. Then, following non-enzymatic and enzymatic reactions, nitrite can be reduced further to nitric oxide (NO), a powerful signalling molecule involved in vasodilation and BP control that lowers blood pressure by 2-5mmHg as a day-to-day homeostatic mechanism [25]. Systematic review however, found no relationship between nitrate reducing bacteria and hypertension in four studies [26], but *Neisseria* species unexpectedly increased in other studies [21, 27]. Limitations included antibacterial mouthwash use and a previous diagnosis of hypertension already being treated with antihypertensive medication [26], both of which may affect the oral microbiome [18, 28]. Participants with undiagnosed hypertension therefore represents a previously unexplored population with potentially less confounders, in which to explore the effects of increased blood pressure on the oral microbiome.

This study therefore employed a dual screening approach, which firstly determined whether dental clinics could repeatedly and reliably case-find patients with undiagnosed hypertension (Study 1). Secondly, it undertook salivary oral microbiome screening in patients not receiving hypertensive medications or mouthwashes, to determine oral microbiome changes in people identified with hypertension for the first time (Study 2); this knowledge being essential prior to any form of personalised oral interventions in this group to improve blood pressure.

MATERIALS AND METHODS

Ethics

This study was approved by the University of Plymouth Human Ethics Committee (#2684) and conducted in accordance with the Human Tissue Act 2004 and GDPR, ensuring ethical standards for tissue handling, confidentiality, and data management. In compliance with ethics procedures, informed consent was obtained from all subjects to allow the anonymised analysis and publication of their personal and medical information, as well as human tissue (saliva).

However, as a cross-sectional, observational study, it was deemed not to be an interventional or randomised controlled trial, and was thus not registered on public clinical trial registries (e.g., clinicaltrials.gov).

Setting

The study was conducted at the Peninsula Dental School (PDS; University of Plymouth, UK) Dental Clinical Education Facility, Devonport (PL1 4JZ), which situated in an area of low socioeconomic status (IMD 2; being among the 20% most deprived neighbourhoods in England) [29]. All patients continued emergency and routine dental treatment by students or staff clinics unaffected by this research study, and the BP and oral microbiome screening took place at a different date and in a different room to the ongoing clinical care.

Inclusion criteria

Between October 2021 and January 2024, 401 adults (>18 years) were approached for recruitment into the study (Figure 1). Following consent, 200 participants (91 male, 109 female) underwent BP screening, BMI assessment, and demographic data. The study comprised two parts: Study 1 (BP screening clinic) and Study 2 (oral microbiome analysis and assessment of periodontal health).

Systemic health: BP, height and weight measurements (BMI)

For Study 1 focussing on systemic health, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), body height and weight (for Body Mass Index; BMI calculation) were all measured as described in our pilot publications [16-17].

Upon arrival to the dental clinic, participants were taken to a quiet room where they completed a health questionnaire including any previous diagnosis of hypertension, hypercholesterolaemia, stroke, heart attack, or diabetes, and a social history including their level of education and current occupation. Participants were then provided correct positioning the appropriate cuff size, with arm and back support compliant with AHA guidelines for accurate measurements [30], alongside a 10-minute rest period, before SBP and DBP were recorded in the left arm using an electronic monitor (ProBP 3400, Welch Allyn, US). Three measurements were taken, and the lowest two measurements were averaged to determine BP and MAP. There was a 1-minute wait between each measurement. Many published studies classify BP for research purposes using similar BP measurements [31-32]. Participants then underwent height and weight measurement using a height measurement scale and weight measurement scale to calculate their BMI using the NHS BMI calculator.

Great care was taken using the average of three BP measurements, using rest periods and being performed at some other time than dental appointments to help avoid white coat syndrome [17]. Participants were then classified in three groups for observational SBP, DBP and MAP detection, according to current NICE guidelines [33-34]: normotensive (NT) (SBP<130mmHg; DBP<80mmHg); prehypertensive (PHT) (SBP=130-139mmHg; DBP 80-89mmHg); and hypertensive (HT) (SBP>140mmHg; DBP>90mmHg). These three clinical group distinctions applied to all participants screened and were important for the observational aspect of this study to inform GP referral [16-17].

For subsequent oral health and oral microbiome analysis in Study 2 (Figure 1), pre-hypertensive participants were excluded from data analysis, to enable comparison between the lowest and highest BP categories (normotensive and hypertensive), thereby highlighting differences associated with disease, rather than mildly elevated BP.

Exclusion criteria

For Study 2 focussing on the oral microbiome, by measuring the oral microbiome and salivary biomarkers alongside periodontal health, the exclusion criteria applied were the factors known to influence the oral microbiome (Figure 1). These included existing diagnosis of diabetes (Type I or II), use of complete dentures, current immunosuppressant therapy, use of antimicrobial mouthwash within the past 3 months, use of antihypertensive medication, being unfasted for 3 hours and consumption of predefined nitrate-rich foods within 48 hours before the appointment [31]. This led to the exclusion of N=161 participants (Figure 1), with N=39 participants undergoing analysis of the salivary microbiome, salivary biochemical markers and periodontal health (NT-OM; SBP<130mmHg; DBP<80mmHg, N=24; HT-OM, SBP>140mmHg; DBP>90mmHg, N=15) [32] (Figure 1). For Study 2, neither group included any participant with a previous history of hypertension or hypertension well controlled with anti-hypertensive medication, as the disease state would be present in these participants and thus may affect the oral microbiome.

Salivary oral microbiome analyses

DNA from saliva pellets were extracted using the DNeasy® PowerSoil® Pro Kit (QIAGEN) and sent to Queen Mary University London Genome Centre (London, UK). Amplification of the 16S rRNA V1-2 region was carried out using universal 16S primers 27F YM (5-AGAGTTTGTATYMTGGCT CAG, where Y is C or T) [35] and 338R (5'-TGCTGCCTCCCGTAGAGT-3') [36]. The libraries were sequenced using the multiplexed, barcoded 300 bp paired end read methodology using the NextSeq2000 platform (Illumina) according to the manufacturer's instructions. Samples were demultiplexed and then exported as fastq files. Bioinformatic analyses were performed on the fastq files using R Studio (V2024.04.0) to remove primers and trim sequences. Low quality ends of the forward and reverse reads were trimmed, and the reads filtered using default parameters (maxN=0; maxEE=2, 4). The filtered reads were then error corrected, merged and chimeras were removed in the DADA2 pipeline (V1.16) [37]. Taxonomy was assigned to the error corrected amplicon sequence variants (ASVs) using the eHOMD database's 16S rRNA training set [37]. Sequence counts of ASVs that mapped to Human Microbial Taxa (HMT) up to genus, species or sub-species levels were agglomerated in *phyloseq*. Read depth was approximately 0.8365 million reads (±87,041.5). To minimise potential sources of bias, standardised protocols were used for sample collection and analysis. As well as differential abundance, we report relative abundance (%) for consistency with previous studies and to enable direct comparison across samples and studies despite variations in total counts or sequencing depth [21]. The datasets generated and/or analysed during the current study are available in the NCBI SRA BioSamples repository. SRA records (PRJNA1465628)

Biochemical analyses

Salivary nitrate, nitrite, glucose, lactate, pH, ammonia were measured by HPLC, IL-6 and IL-10 were analysed using ELISA; via our previous protocols already published in the literature [28, 32, 38].

Periodontal health measurements

These data were taken from examinations were carried out by dental or dental therapy students under the supervision of a General Dental Council (GDC)-registered dentist, at a later time (within 6 weeks) to the BP screening clinic. A qualified GDC-registered dental practitioner (Level 2; Specialist Interest in Periodontology with Postgraduate training) further reviewed six-point pocket charts (PPD and CAL) and relevant radiographs to classify periodontal disease severity according to CDC/AAP case definitions into no periodontitis, mild, moderate or severe [39], ensuring international comparability of findings.

Statistical Analysis

Normal distribution of data was assessed using the Shapiro-Wilk test. Differences between >2 groups (NT, PHT and HT) were analysed using one-way ANOVA (normally distributed) or a Kruskal Wallis test (non-normally distributed). Differences between 2 groups (NT and HT) were analysed using independent t-tests (normally distributed) or a Mann-Whitney test (non-normally distributed). Categorical variables were assessed with Chi-Squared. Multiple linear regression analyses were carried out using XLSTAT and Excel to determine the impact of salivary biomarkers and demographic variables on SBP and DBP. Basic data analysis was conducted using Past4 (V4.16) for statistical analysis. Regarding microbial analyses, alpha and beta diversity were examined using Past4 (V4.16). Alpha diversity was visualized using Shannon index (H) boxplots and compared with Mann-Whitney tests (Bonferroni-corrected). Beta diversity was visualized with NMDS plots based on Bray-Curtis distances and compared using one-way PERMANOVA with Bonferroni correction. ONRB used for assessment of relative abundance between NT and HT were derived from Rosier et al., [40]. The application of relative abundance for this type of analysis has been well documented in prior studies [21, 41]. Differential abundance analysis of oral bacteria was also carried out using ANCOM-BC analysis (R Studio, V2024.04.0). Significantly differentially abundant taxa between groups were represented using forest plots representing log fold changes (R Studio, V2024.04.0).

RESULTS

Study 1 Blood Pressure Screening (N=200 participants):

Normotensive: N=87 (43.5%) participants were measured as having normotensive (NT) blood pressures (Table 1). Of the N=87 participants measured with NT, N=17 had a prior clinical diagnosis of hypertension that was successfully managed with medication or lifestyle changes. No GP referrals were needed.

Pre-hypertensive: N=50 (25%) participants were measured as having pre-hypertensive (PHT) blood pressures (data not shown). Of the N=50 PHT participants, N=29 had no prior clinical diagnoses of hypertension and were thus not receiving any form of hypertensive treatment, while N=21 had a prior clinical diagnosis of hypertension and were receiving antihypertensive medication. No GP referrals were provided.

Hypertensive: N=63 (31.5%) participants were measured as having hypertensive (HT) blood pressures overall (Table 1). Of these N=63 HT participants with increased blood pressure, N=37 were diagnosed for the first time in this study (previously undiagnosed) and N=26 had a prior diagnosis of hypertension for which they already received medication or lifestyle modification.

GP referral: Of the N=26 known HT patients receiving possibly 'unsuccessful' anti-hypertensive medication, N=3 underwent medication modifications via their GP after referral via this study.

Of the N=37 participants measured with hypertensive blood pressure for the first time and referred to their GP, only N=9 succeeded in obtaining a GP appointment. Following this appointment, their 'hypertension' diagnosis was confirmed (4.5%). Out of these N= 9 participants confirmed to have hypertension, N=7 then received antihypertensive medication and N=1 was hospitalised for 13 days after follow-up with the GP for heart failure and suspected acute coronary syndrome [16].

Height and weight (BMI): Among the N=200 participants attending BP screening clinics, N=5 (2.5%) had a low BMI (<20kg/m²), N=49 (24.5%) had a normal BMI (20–24.9kg/m²) and N=76 (38.0%) had a high BMI (>3 kg/m²). HT participants had significantly higher BMIs than NT participants (NT=27.09±5.68; HT=30.62±6.01, P<0.001).

Systemic health correlation analysis: Using all N=200 participants, a multiple linear regression analysis was performed to evaluate the impact of demographic variables on SBP and DBP. The independent predictors included gender, age, body mass index (BMI), and smoking status. The model accounted for 15.8% of the variance in SBP, with an adjusted R² of 0.158 (F[4,199]=10.33, P<0.0001). Specifically, male gender (t=2.363, P=0.019), higher BMI (t=4.328, P<0.001) and older age (t=3.385, p<0.008) were confirmed as significant contributors, as all were associated with increased SBP. The model accounted for 6.4% of the variance in DBP, with an adjusted R² of 0.064 (F[4,199]=4.407, P=0.001). Specifically, male gender (t=3.358, P=0.0009), was identified as a significant contributor, both associated with increased SBP.

Undiagnosed hypertensive: After applying the exclusion criteria described in the methods (Figure 1) to the N=37 previously undiagnosed HT participants, N=15 remained with blood pressure measured as hypertensive (Table 2). These N=15 participants with reduced confounding factors and no anti-hypertensive medication were used for oral microbiome screening (HT-OM).

Salivary oral microbiome: Considering overall abundance, no significant differences were observed in α -diversity between NT-OM and HT-OM groups (NT-OM: 3.72 ± 0.30 N=24; HT-OM: 3.59 ± 0.36 N=15; $P=0.252$), nor in β -diversity (Stress=0.1808; $F=1.285$; $P=0.206$) (Figure 2A and B).

However, at phyla level taxonomic analysis revealed that the relative abundance of the phylum *Actinomycetota* was significantly higher in HT-OM ($20.44\% \pm 4.92\%$ N=24; $P=0.015$) compared to NT-OM ($16.50\% \pm 4.70\%$ N=15) (Figure 2C). In contrast, the phylum *Fusobacteriota* was less abundant in HT-OM ($2.31\% \pm 1.13\%$; $P<0.050$) than NT-OM ($4.17\% \pm 3.65\%$) (Fig 2C).

At genera level, *Alloscardia*, *Anaeroglobus*, *Dialester*, *Olsenella*, *Peptiphaga*, *Saccharibacteria* and *Veillellaceae* all increased in HT compared to NT (Table 3). However, there were no significant differences in the abundance of the *Neisseria* genus overall (NT-OM = 2.81 ± 3.10 ; HT-OM = 1.71 ± 2.80 , $P>0.05$) (Figure 2D).

Salivary oral microbiome and ONRB: At species level, the HT-OM group had increased relative abundance of ONRB species overall (NT-OM, $17.45\% \pm 5.94\%$ N=24; HT-OM, $21.88\% \pm 6.27\%$ N=15; $P=0.031$). Specifically, there was increased relative abundance of *Actinomyces massiliensis* in the HT-OM group (NT-OM, $0.02\% \pm 0.02\%$; HT-OM, $0.04\% \pm 0.04\%$; $P=0.012$) (Figure 2E). The ONRB *Neisseria flavescens* was also increased in the HT-OM group, but the ONRB *Neisseria subflava* was lower in the HT-OM group (Figure 2F).

Salivary biochemical markers: Results are presented in Table 2. Salivary ammonia was significantly lower in HT-OM compared to NT-OM ($P<0.01$). Also, ONRA was significantly higher in HT-OM compared to NT-OM ($P<0.05$), but all other parameters did not differ between groups.

Periodontal health: Periodontitis diagnoses were identified in the N=24 NT-OM and N=15 HT-OM participants with available oral health data. Categorising periodontitis into mild, moderate and severe resulted in small numbers with no significant differences between HT-OM and NT-OM participants (Table 2) ($\chi^2(2)=3.67$, $P=0.160$). Assessment of no periodontitis versus any periodontitis, further demonstrated a no significant differences between BP groups ($p=0.095$).

Correlation analyses: Using all N=24 NT-OM and N=15 HT-OM participants, a multiple linear regression analysis was performed to evaluate the relationship between SBP and demographic variables, periodontal health or salivary biomarkers. The independent predictors of BP included periodontitis diagnosis, salivary concentrations of nitrate, nitrite, ONRA, ammonia, lactate, glucose, pH, buffering capacity, and inflammatory markers (IL-6 and IL-10), along with covariates such as sex, age, body mass index (BMI), and smoking status. The model accounted for 47.2% of the variance in SBP, with an adjusted R^2 of 0.472 ($F[15,38]=3.261$, $P=0.005$). Specifically, higher BMI ($t=2.238$, $P=0.035$), being male ($t=-2.851$, $P=0.009$) and increased salivary glucose ($t=2.166$, $P=0.040$) were identified as significant predictors, associated with increased SBP.

A similar regression model was applied to assess the impact of the same predictors on DBP. This model explained 23.3% of the variance in DBP, with an adjusted R^2 of 0.233 ($F[15,38]=1.770$, $P=0.105$). Within this model, increased salivary glucose ($t=2.323$, $P=0.029$) was associated with increased DBP, supported by our Pearson's correlation analyses showing a significant positive correlation ($r=0.315$; $P=0.025$). Regression analysis did not identify any correlation between periodontitis diagnosis and SBP or DBP.

DISCUSSION

To the best of our knowledge, this is the first study setting up blood pressure screening within a primary care dental clinic including salivary microbiome/biochemical analysis as part of the overall assessment of participant's oral and systemic health. Despite seemingly accurate blood pressure measurements, it identified challenges obtaining follow-up care for patients with hypertension, when attempting a preventive and integrated primary care-based approach. The model, however, did allow the oral microbiome to be assessed in participants with previously undiagnosed high blood pressure at some other time than dental treatment, without confounding factors such as anti-hypertensive medications and mouthwash use. This was important to consider as many previous studies have shown conflicting responses, perhaps due to the inclusion of confounding factors. The data produced here thus allowed both the abundance and activity of bacteria to be assessed within the oral microbiome, revealing species level differences in nitrate reducing bacteria that may be relevant for future preventative or screening approaches in hypertensive patients.

In terms of the initial blood pressure screening, our study found that 31.5% participants had raised blood pressures indicative of hypertension overall. This agreed with 32% reported in a Swedish blood screening study involving >1,000 participants [42], but below a Kuwaiti study reporting 50.1% [43]. Our protocol involved a measurement taken at some other time than the dental appointment, and strict adherence to guideline protocols to eliminate 'white coat' syndrome [14], which may have explained this. However, out of 37 at risk patients, only 9 saw their GP due to either: not being interested in follow-up, not being able to get a GP appointments or GPs rejecting our referral because it was felt this initiative was increasing GP workload. Such challenges with referrals and access to a GP suggest that for 'non-medical' BP screening initiatives to succeed on a larger scale, more needs to be done regarding the development of integrated care pathways and improved access to care for patients, especially in areas of low socioeconomic status, where access to medical and dental treatment is a particular challenge [44]. However, confirmation of a hypertensive diagnosis in 7 out of 9 cases by GP's, when participants were able to get appointments as follow up, reassured us that our careful protocols for blood pressure measurement were effective in the majority of cases.

As our initial literature review had suggested varying responses of the oral microbiome among different hypertensive populations, thus as a new approach, we next eliminated confounders factors in undiagnosed hypertensive participants, before proceeding to oral microbiome analysis. We are not aware of any previous studies investigating the oral microbiome in undiagnosed hypertensive populations prior to the use of medications or lifestyle modifications. It was interesting therefore, to find no overall change in the abundance of bacteria during hypertension in agreement with previous studies [26]. When being more specific and considering the presence or absence of 'health'-related bacteria in hypertensive participants, ONRB abundance increased. This was not what was anticipated, considering blood pressure increased, but was also agreement with previous studies [21]. This included for example, *Neisseria flavescens* - a

strong nitrate reducer [45-47], being increased in hypertensive participants as previously observed [26, 48-49]. Conversely however, *Neisseria subflavens* species decreased and our genus and phylum data encompassing various nitrate reducers, offered mixed patterns of responses, as previously seen [26]. The conflicting responses among nitrate reducing species such as *Neisseria*, were identified here via a deeper dive using ANCOM-BC. In this study we also observed that *Veillellaceae* genera increased during hypertension and, at species level, previous studies have shown that *Veillonella* species can have beneficial roles, such as nitrate reduction, even though *Veillonella* are sometimes associated with periodontitis [40,41]. Such complex, and sometimes contradictory functions among oral microbiome species therefore, confirms that targeting specific bacteria within co-dependent communities, might not be the best treatment approach until more is known about functionality at species level, as well as overall abundance and activity. Alongside the oral microbiome, additional nitrite production from host immune-cell activity during inflammation has also not been considered by ourselves, or fully by others, in this context [50]. This study has therefore identified that current practices using oral microbiome screening to guide personalised oral hygiene measures, with a goal of managing systemic diseases, remain somewhat premature at this time. Such clinical practices therefore need further research to support them.

Further, in terms of the oral host microenvironment, a moderate positive correlation was observed here between salivary glucose and systolic BP. Although salivary glucose is not routinely used to assess glycaemic status, it can indicate systemic metabolic changes such as insulin resistance [51]. Elevated salivary glucose may also reflect microbial dysbiosis, as glucose fuels acidogenic bacteria like *Streptococcus mutans* and *Lactobacillus species* [52]. In this study *Actinomycetota* phyla was significantly higher in the hypertension group, broadly aligning with links between hypertension, dental caries, and some *Actinomyces species* being lactic acid-producing pathogens. Given the link between periodontitis and poor glycaemic control [53-54], this possible correlation further supports current dental best practice guidelines recommending that patients with diabetes are a priority for improved oral hygiene, as periodontitis leads to poor blood glucose control [55-56].

One limitation of our study was that we did not observe increased periodontitis in the hypertensive group, an association which has previously been confirmed via systematic review [4]. This may have been due to our challenging demographics, being positioned in an area of low socioeconomic status where the overall incidences of periodontitis (64%), smoking (24.0%) and BMI (28.1) were much higher than the UK national average [57-59]; in essence they had multiple issues of poor systemic health other than hypertension. Undiagnosed diabetes was possibly another confounder; even though known diabetic patients were excluded, diabetic screening would have been useful, as there may have been many undiagnosed cases among our less healthy participants. Furthermore, applying oral microbiome screening after BP screening to investigate a specific undiagnosed population and produce more robust data, also meant that the sample size reduced. However, similar published studies on the oral microbiome have used fewer participants [60]. Future studies could now apply such confounding factors described here prospectively, as part of conducting larger studies with increased sample size.

Regarding other future directions, our data suggest that oral nitrate reducing bacteria remain of interest in people with high blood pressure. Analysis of the oral microbiome from newly hypertensive individuals without antihypertensive medications and mouthwashes agreed with, for example, increases in some *Neisseria* species during hypertension [21]. This is important to confirm, as such nitrate usually decrease blood pressure in healthy people and therefore, this response is the opposite response to what one would expect. Perhaps without intervention early in hypertension the oral microbiome tries to compensate to protect the host? Research has alluded to similar compensatory mechanisms of the gut through hormones and/or enzymes [61-62]. This could therefore emerge as a similar possibility in the oral cavity and we await further studies before any firm conclusions can be made [63].

In conclusion, blood pressure screenings in dental clinics, at a time other than dental treatment, can reliably identify people with undiagnosed hypertension, but more work needs to be done with integrated care pathways and patient access to care for them to be fully functional. Hypertension can also be linked to changes in the oral microbiome including both increases and decreases in nitrate reducing species, suggesting that bacteria may behave very different at species level. Thus, although 'balancing' the oral microbiome has huge potential for restoring oral health and in turn cardiovascular health, because of the differing functions of different bacterial species, and even beneficial and detrimental among the same species (depending on the environment), there is still much to learn. Thus, whilst oral hygiene remains the cornerstone of oral disease prevention, at this time we urge oral healthcare professionals to be extremely cautious of using oral microbiome screening and metagenomics to identify single bacteria using metagenomics approaches, in order to develop treatment plan and personalised oral hygiene treatments.

Declarations

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Conflicts of interest: NONE

Data Availability: The datasets generated and/or analysed during the current study are available in the NCBI SRA BioSamples repository. SRA records (PRJNA1465628)

References

1. NHS. Core20PLUS5 infographic – Adult [Internet]. NHS; 2022. Available from: <https://www.england.nhs.uk/publication/core20plus5-infographic/>. Accessed February 15, 2023.
2. Raizada M, Joe B, Bryan N, et al. Report of the National Heart, Lung, and Blood Institute Working Group on the role of microbiota in BP regulation: current status and future directions. *Hypertension*. 2017;70:479–485.
3. Charchar FJ, Prestes PR, Mills C, et al. Lifestyle management of hypertension: International Society of Hypertension position paper endorsed by the World Hypertension League and European Society of Hypertension. *J Hypertens*. 2024;42(1):23–49.
4. Muñoz Aguilera E, Suvan J, Orlandi M, et al. Association between periodontitis and blood pressure highlighted in systemically healthy individuals: results from a nested case-control study. *Hypertension*. 2021;77(5):1765–1774.
5. Rodrigues JVS, Cláudio MM, Franciscan JPS, et al. The effect of non-surgical periodontal treatment on patients with combined refractory arterial hypertension and stage III, grade B periodontitis: a preliminary prospective clinical study. *J Clin Med*. 2023;12(13):4277.
6. Lanau N, Mareque-Bueno J, Zabalza M. Impact of nonsurgical periodontal treatment on blood pressure: a prospective cohort study. *Eur J Dent*. 2024;18(2):517–525. doi:10.1055/s-0043-1772246.
7. World Health Organization. First WHO report details devastating impact of hypertension and ways to stop it [Internet]. 2023. Available from: <https://www.who.int/news/item/19-09-2023-first-who-report-details-devastating-impact-of-hypertension-and-ways-to-stop-it>. Accessed October 30, 2025.
8. World Health Organization. Uncontrolled high blood pressure puts over a billion people at risk [Internet]. 2025. Available from: <https://www.who.int/news/item/23-09-2025-uncontrolled-high-blood-pressure-puts-over-a-billion-people-at-risk>. Accessed October 30, 2025.
9. Stergiou GS, Kyriakoulis KG, Kollias A, et al. Blood pressure measurement at kiosks in public spaces: systematic review and consensus statement by the European Society of Hypertension Working Group on Blood Pressure Monitoring and Cardiovascular Variability endorsed by the International Society of Hypertension and the World Hypertension League. *J Hypertens*. 2025;43(4):577–588.
10. Jones DW, Ferdinand KC, Taler SJ, Johnson HM, Shimbo D, Abdalla M, Altieri MM, Bansal N, Bello NA, Bress AP, et al. 2025 AHA/ACC/AANP/AAPA/ABC/ACCP/ACPM/AGS/AMA/ASPC/NMA/PCNA/SGIM guideline for the prevention, detection, evaluation and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation*. 2025;86:1567.
11. Stergiou GS, Palatini P, Parati G, O'Brien E, Januszewicz A, Lurbe E, Persu A, Mancia G, Kreutz R, et al. 2021 European Society of Hypertension practice guidelines for office and out-of-office blood pressure measurement. *J Hypertens*. 2021;39(7):1293–1302.
12. Zeniodi ME, Tsaganos T, Menti A, Komnianou A, Kollias A, Stambolliu E. European Hypertension Guidelines: Similarities and What the Practicing Physician Should Keep in Mind. *J Clin Med*. 2026;15(2):859.
13. Pioli MR, Ritter AM, de Faria AP, Modolo R. White coat syndrome and its variations: differences and clinical impact. *Integr Blood Press Control*. 2018;11:73–79.
14. Andersson H, Hedström L, Bergh H. White-coat hypertension detected during opportunistic blood pressure screening in a dental healthcare setting. *Scand J Prim Health Care*. 2021;39(3):348–354.
15. Huang Y, Huang W, Mai W, Cai X, An D, Liu Z, Huang H, Zeng J, Hu Y, Xu D. White-coat hypertension is a risk factor for cardiovascular diseases and total mortality. *J Hypertens*. 2017;35(4):677–688.
16. Doble A, Bescos R, Witton R, et al. Uncontrolled hypertension at the dentist: a case report of integrated healthcare. *Br Dent J*. 2023;235:866–868.
17. Doble A, Bescos R, Witton R, et al. A case-finding protocol for high cardiovascular risk in a primary care dental school - model with integrated care. *Int J Environ Res Public Health*. 2023;20:4959.
18. Al-Maweri SA, Al-Mashraqi AA, Al-Qadhi G, et al. The association between the oral microbiome and hypertension: a systematic review. *J Oral Microbiol*. 2025;17(1):2459919.
19. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K. Oral Microbiome: A Review of Its Impact on Oral and Systemic Health. *Microorganisms*. 2024;12(9):1797.
20. British Society of Periodontology and Implant Dentistry. Oral microbiome testing – BSP statement & fact sheet [Internet]. 2025. Available from: https://www.bsperio.org.uk/assets/downloads/Oral_Microbiome_Testing_-_BSP_Statement___Fact_Sheet.pdf. Accessed April 15, 2026.
21. Sohail MU, Hedin L, Al-Asmakh M. Dysbiosis of the salivary microbiome is associated with hypertension and correlated with metabolic syndrome biomarkers. *Diabetes Metab Syndr Obes*. 2021;14:4641–4653.
22. Sulaiman Y, Pacauskienė IM, Šadzevičienė R, Anuzyte R. Oral and gut microbiota dysbiosis due to periodontitis: systemic implications and links to gastrointestinal cancer—a narrative review. *Medicina (Kaunas)*. 2024;60(9):1416.
23. Brookes Z, Teoh L, Cieplik F, Kumar P. Mouthwash effects on the oral microbiome: are they good, bad, or balanced? *Int Dent J*. 2023;73(Suppl 0):S74–S81.
24. Chai X, Liu L, Chen F. Oral nitrate-reducing bacteria as potential probiotics for blood pressure homeostasis. *Front Cardiovasc Med*. 2024;11:1337281.
25. Hopkins S, Gajagowni S, Qadeer Y, et al. More than just teeth: how oral health can affect the heart. *Am Heart J Plus*. 2024;43:100407.
26. Puel EM, Taruhn LF, Damé-Teixeira N, Stefani CM, Lатарo RM. Is there a link between the abundance of nitrate-reducing bacteria and arterial hypertension? A systematic review. *Nitric Oxide*. 2025;157:19–33.
27. Chen BY, Lin WZ, Li YL, et al. Roles of oral microbiota and oral-gut microbial transmission in hypertension. *J Adv Res*. 2023;43:147–161.
28. Bescos R, Ashworth A, Cutler C, et al. Effects of chlorhexidine mouthwash on the oral microbiome. *Sci Rep*. 2020;10(1):5254.

29. Ministry of Housing, Communities & Local Government. Local Deprivation Explorer (English indices of deprivation 2025) [Internet]. 2025. Available from: <https://deprivation.communities.gov.uk/>. Accessed April 15, 2026.
30. Foti K, Moran AE, Matsushita K, Appel LJ, Juraschek SP, Pathni AK, Banigbe BF, Dessie GA, Belbase B, Bhuiyan MR, et al. Evidence-based, streamlined approach to measure blood pressure in primary care settings. *Hypertension*. 2026;83(2):e24527.
31. Ashworth A, Bescos R. Dietary nitrate and blood pressure: evolution of a new nutrient? *Nutr Res Rev*. 2017;30(2):208–219.
32. Bescos R, du Toit L, Redondo-Rio A, et al. The comparative effect of propolis and chlorhexidine mouthwash on oral nitrite-producing bacteria and blood pressure regulation. *J Oral Microbiol*. 2024;17(1):2439636.
33. National Institute for Health and Care Excellence (NICE). Hypertension in adults: diagnosis and management [Internet]. NICE; 2022. Available from: <https://www.nice.org.uk/guidance/ng136>. Accessed April 4, 2023.
34. NHS Digital. Health Survey for England, 2019: Adults Health Report [Internet]. Leeds, UK: NHS Digital; 2020. Available from: <https://files.digital.nhs.uk/23/6B5DEA/HSE19-Adult-health-rep.pdf>. Accessed February 22, 2024.
35. Frank J, Reich C, Sharma S, et al. Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Appl Environ Microbiol*. 2008;74(8):2461–2470.
36. Wade W, Prosdocimi E. Profiling of oral bacterial communities. *J Dent Res*. 2020;99(6):621–629.
37. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13:581–583.
38. Ashworth A, Cutler C, Farnham G, et al. Dietary intake of inorganic nitrate in vegetarians and omnivores and its impact on blood pressure, resting metabolic rate and the oral microbiome. *Free Radic Biol Med*. 2019;138:63–72.
39. Pietropaoli D, Del Pinto R, Ferri C, et al. Poor oral health and blood pressure control among US hypertensive adults. *Hypertension*. 2018;72(6):1365–1373.
40. Rosier BT, Johnston W, Carda-Diéguez M, et al. Nitrate reduction capacity of the oral microbiota is impaired in periodontitis: potential implications for systemic nitric oxide availability. *Int J Oral Sci*. 2024;16(1):1.
41. Burleigh MC, Liddle L, Monaghan C, et al. Salivary nitrite production is elevated in individuals with a higher abundance of oral nitrate-reducing bacteria. *Free Radic Biol Med*. 2018;120:80–88.
42. Engström S, Berne C, Gahnberg L, et al. Efficacy of screening for high blood pressure in dental health care. *BMC Public Health*. 2011;11:194.
43. Abdulwahab M, Kamal M, Akbar A. Screening for high blood pressure at the dentist's office. *Clin Cosmet Investig Dent*. 2022;14:79–85.
44. Plymouth City Council. Plymouth oral health needs assessment summary [Internet]. 2019. Available from: <https://democracy.plymouth.gov.uk/documents/s100891/Oral%20Health%20Needs%20Assessment%20Summary%202019.pdf>. Accessed March 20, 2026.
45. Rosier B, Takahashi N, Zaura E, et al. The importance of nitrate reduction for oral health. *J Dent Res*. 2022;101(8):887–897.
46. Velmurugan S, Gan J, Rathod K, et al. Dietary nitrate improves vascular function in patients with hypercholesterolemia: a randomized, double-blind, placebo-controlled study. *Am J Clin Nutr*. 2016;103(1):25–38.
47. Hyde ER, Andrade F, Vaksman Z, et al. Metagenomic analysis of nitrate-reducing bacteria in the oral cavity: implications for nitric oxide homeostasis. *PLoS One*. 2014;9:e88645.
48. Barbadoro P, Ponzio E, Coccia E, et al. Association between hypertension, oral microbiome and salivary nitric oxide: a case-control study. *Nitric Oxide*. 2021;106:66–71.
49. LaMonte MJ, Gordon J, Diaz-Moreno P, et al. Oral microbiome is associated with incident hypertension among postmenopausal women. *J Am Heart Assoc*. 2022;11(6):e021930.
50. Bescos R, Gallardo-Alfaro L, Ashor A, et al. Nitrate and nitrite bioavailability in plasma and saliva: their association with blood pressure—a systematic review and meta-analysis. *Free Radic Biol Med*. 2025;226:70–83.
51. Gupta V, Kaur A. Salivary glucose levels in diabetes mellitus patients: a case-control study. *J Oral Maxillofac Pathol*. 2020;24(1):187.
52. Rehman K, Akash MSH. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci*. 2016;23:87.
53. Preshaw PM, Alba AL, Herrera D, et al. Periodontitis and diabetes: a two-way relationship. *Diabetologia*. 2012;55(1):21–31. doi:10.1007/s00125-011-2342-y.
54. British Society of Periodontology and Implant Dentistry. Gum disease and diabetes [Internet]. 2025. Available from: <https://www.bsperio.org.uk/patients/gum-disease-and-diabetes>. Accessed November 18, 2025.
55. British Society of Periodontology and Implant Dentistry. Diabetes guide [Internet]. 2025. Available from: <https://www.bsperio.org.uk/news/diabetes-guide>. Accessed April 15, 2026.
56. British Society of Periodontology and Implant Dentistry. Gum health awareness for health care professionals - diabetes [Internet]. 2025. Available from: https://www.bsperio.org.uk/assets/downloads/Diabetes_and_gum_disease_information_for_health_care_professionals.pdf. Accessed April 15, 2026.
57. NHS Digital. Health Survey for England 2022: adult overweight and obesity [Internet]. 2024. Available from: <https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/2022-part-2/adult-overweight-and-obesity>. Accessed March 20, 2026.
58. Office for National Statistics. Adult smoking habits in Great Britain: 2024 [Internet]. 2025. Available from: <https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/healthandlifeexpectancies/bulletins/adultsmokinghabitsingreatbritain/20>. Accessed March 20, 2026.

59. Office for Health Improvement and Disparities. Adult oral health survey 2023: clinical oral health [Internet]. 2025. Available from: <https://www.gov.uk/government/statistics/adult-oral-health-survey-2023/clinical-oral-health#periodontal-disease-1>. Accessed March 20, 2026.

60. du Toit L, Offiah I, Redondo-Rio A, Kahawita T, Kiernan M, Lin YM, Belfield L, Montagut G, Benavente A, Nicholas T, Doble A, Dikkers R, Freeman R, Gabaldón T, Brookes Z, Bescos R. A pilot and feasibility study investigating the abundance and activity of nitrate-reducing bacteria in women with pre-eclampsia. *Pregnancy Hypertens.* 2025;39:101188.

61. Desai M, Seekatz A, Koropatkin N, Kamada N, Hickey C, Wolter M, et al. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell.* 2016;167(5):1339.e21–1353.e21.

62. Martin A, Sun E, Rogers G, Keating D. The influence of the gut microbiome on host metabolism through the regulation of gut hormone release. *Front Physiol.* 2019;10:428.

63. Chen BY, Lin WZ, Li YL, et al. Roles of oral microbiota and oral-gut microbial transmission in hypertension. *J Adv Res.* 2023;43:147–161.

Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures

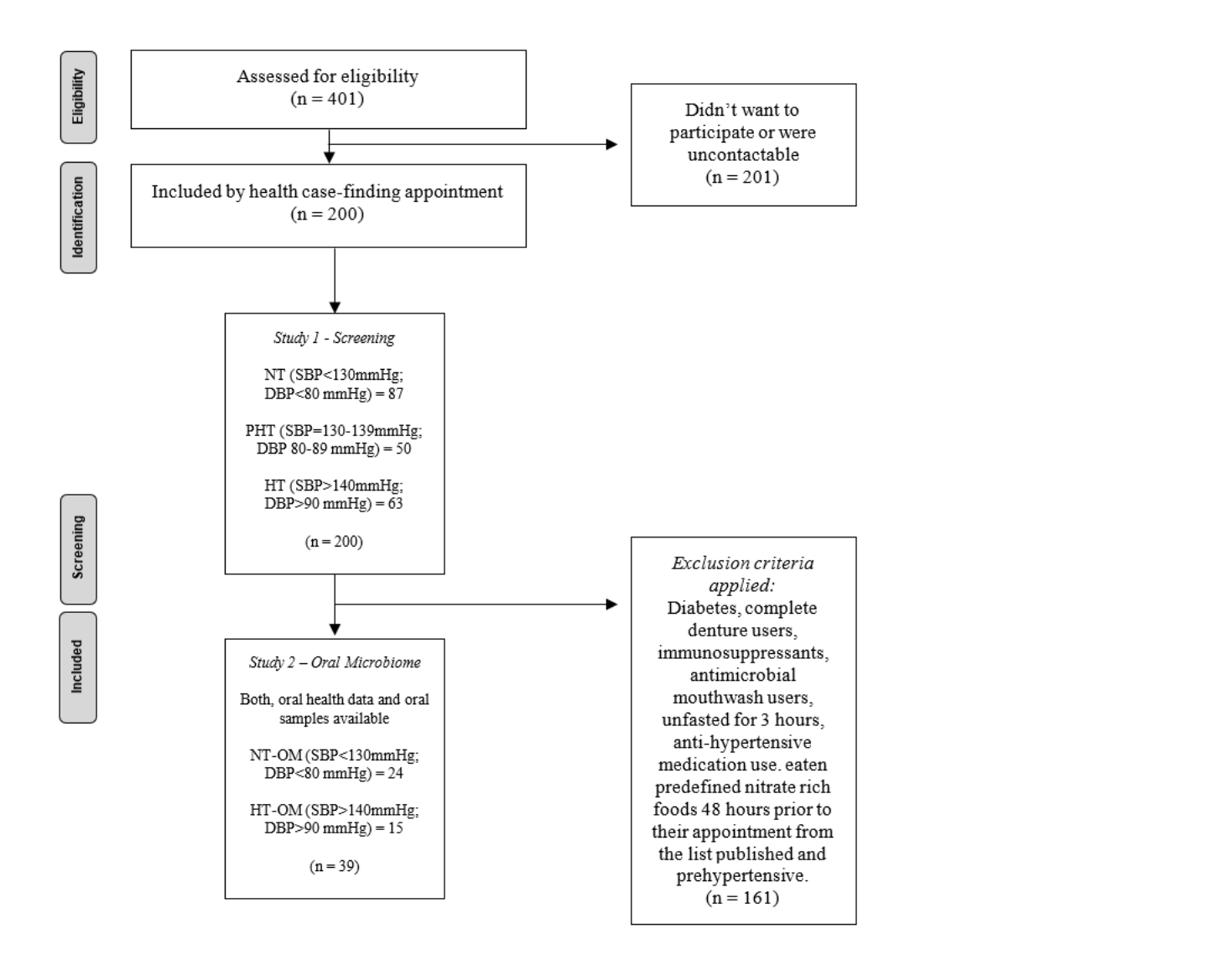


Figure 1

Characteristics of participants assessed for study eligibility (N=401), who then attended the Study 1 research BP screening clinic in the Peninsula Dental School primary care dental setting (N=200). The BP screening occurred at some other time than routine clinical dental appointments, and identified people as either normotensive (NT), pre-hypertensive (PHT; not used for subsequent analysis) or hypertensive (HT). Exclusion criteria were then applied, which removed N=161

participants for Study 2 and resulted in NT-OM (N=24) and HT-OM (N=15) groups that were used for subsequent periodontal health assessment, salivary oral microbiome and salivary biochemical marker analysis.

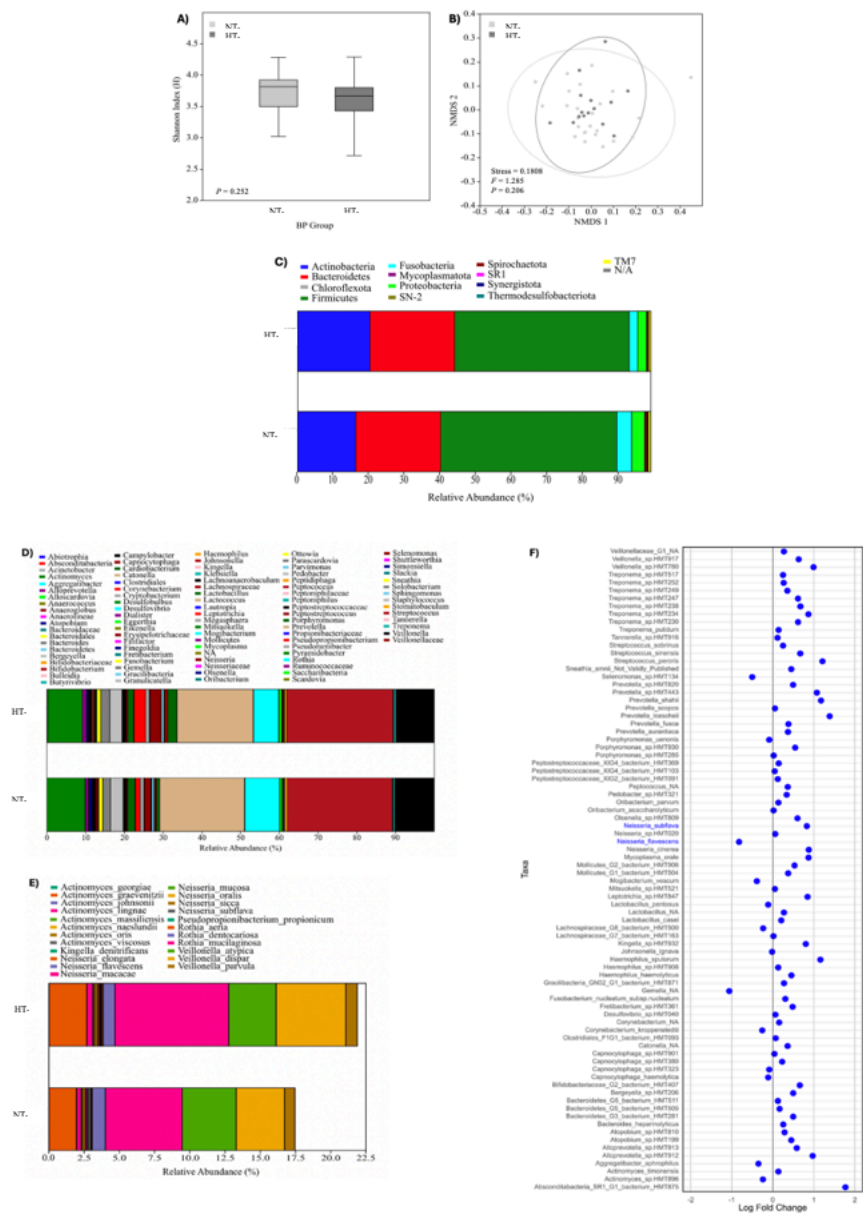


Figure 2

Oral microbiome analysis. Study 2 NT-OM (N=24) and HT-OM (N=15) blood pressure groups after application of exclusion criteria. A) Alpha diversity differences between the NT-OM and HT-OM groups, B) Beta diversity differences between the NT-OM and HT-OM groups, C) Phylum analysis between the NT-OM and HT-OM groups, D) Genera analysis between the NT-OM and HT-OM groups, E) Total ONRB species analysis between the NT-OM and HT-OM groups and F) ANCOM-BC differential abundances between the NT-OM and HT-OM groups. Positive log fold changes represent increased abundance in the NT group. Asterisks in each plot indicate the P-value; * <0.05 , ** <0.01 and *** <0.001 .

Supplementary Files

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- Table.docx