



Original Article

Comparative Analysis of Oral Bacterial Load and Antibiotic Susceptibility Profiles in Libyan Type 2 Diabetes Mellitus (T2DM) and Periodontitis Cohorts

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Abstract

Background: Type 2 diabetes mellitus (T2DM) and periodontitis are prevalent inflammatory conditions interacting bidirectionally. Although genomic alterations have been documented, it remains unclear how early-onset T2DM and periodontitis modify culturable bacterial load and antibiotic hypersensitivity patterns in plaque biofilms in the Libyan population. **Methods:** We conducted a cross-sectional culturing study using supragingival and subgingival plaque from 60 adults (30–40 years) in Benghazi, divided into healthy (H, n=10), periodontitis (P, n=10), T2DM (n=20), and T2DM with periodontitis (T2DM_P, n=20). Total culturable aerobic and anaerobic bacterial loads were determined via colony-forming units (CFU/mg). Antibiotic hypersensitivity profiling was evaluated using the Kirby-Bauer disk diffusion method against amoxicillin, metronidazole, clindamycin, and doxycycline. **Results:** The T2DM_P group exhibited the highest total anaerobic bacterial load ($7.84 \pm 0.42 \log_{10}$ CFU/mg) compared to the healthy group ($4.12 \pm 0.28 \log_{10}$ CFU/mg; $p < 0.001$). Antibiotic hypersensitivity testing revealed significantly reduced zones of inhibition (increased resistance) to metronidazole and amoxicillin in plaque isolates from the T2DM_P and P groups compared to healthy individuals ($p < 0.01$). Clindamycin and doxycycline retained the highest overall hypersensitivity zones across all cohorts. **Conclusion:** Early-onset T2DM combined with periodontitis significantly intensifies the total pathogenic bacterial load and reduces antibiotic susceptibility profiles within dental plaque. Culturable monitoring provides a pragmatic window for targeted local medical-dental interventions.

Keywords: Oral microbiology; Bacterial load; Colony forming units; Antibiotic hypersensitivity; Type 2 diabetes mellitus; Periodontitis

Introduction

Type 2 diabetes mellitus (T2DM) and periodontitis are widespread chronic diseases that share an intricate, bidirectional immuno-inflammatory link [1]. Persistent hyperglycemia impairs immune defences, resulting in altered neutrophil chemotaxis, increased advanced glycation end-products (AGEs), and exaggerated hyper-inflammatory cytokine production, such as interleukin-1 beta (IL-1 β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF- α) [1,2]. This dysregulated systemic landscape actively triggers structural changes within oral mucosal surfaces and the supportive periodontal apparatus [3].

Concurrently, the microenvironment of the oral cavity undergoes prominent functional adaptations. Local shifts in glucose availability and inflammatory exudates filter microflora selection, driving the proliferation of pathogenic complexes within plaque biofilms [4]. While high-throughput genetic sequencing yields comprehensive taxonomic lists, culture-based processing provides direct clinical insights regarding viable organism absolute density (bacterial load) and realistic drug response behaviours via antibiotic hypersensitivity testing [5].

Antibiotic resistance is an escalating public health threat in Libya, heavily impacting standard odontogenic treatment paths. Systemic exposures and repetitive empiric prescribing alter the baseline sensitivity profiles of common therapeutic choices such as penicillins, lincosamides, and nitroimidazoles [6]. Evaluating antibiotic hypersensitivity directly alongside absolute quantitative bacterial densities provides clear criteria for choosing targeted local and systemic adjunct therapies [7]. This cross-sectional pilot study was designed to systematically map viable bacterial loads and antibiotic susceptibility baselines among an early-onset Libyan group of adults. By screening healthy, diabetic, and periodontitis patients, we evaluated how metabolic stress correlates with phenotypic drug susceptibility phenotypes inside plaque microenvironments [8].

Material and Methods

Cohort Recruitment and Criteria

A total of 60 adult participants aged 30–40 years were cross-sectionally recruited from the Government Dental Hospital, Benghazi, Libya. Approval was obtained from the Institutional Ethics Committee (Ref: 7/2025), and all





participants provided written informed consent. Subjects were categorized into four distinct study blocks based on the guidelines of the World Workshop on the Classification of Periodontal Diseases (9): Healthy Controls (H, $n = 10$; normal metabolic parameters, no attachment loss), Periodontitis Patients (P, $n = 10$; clinical attachment loss ≥ 3 mm in multiple sites), T2DM patients without clinical periodontitis (T2DM, $n = 20$; established HbA1c $> 7\%$), and T2DM patients presenting with concurrent Periodontitis (T2DM_P, $n = 20$).

Plaque Collection and Quantifying Bacterial Load

Supragingival plaque was harvested from healthy and diabetic groups via clinical scaling from buccal tooth surfaces [10]. Subgingival plaque was collected from periodontitis and comorbid groups using sterile curettes targeting the deepest periodontal pockets with a probing depth ≥ 5 mm [11]. Samples were weighed and immediately transferred into 1 mL sterile phosphate-buffered saline (PBS) solution. Serial log tenfold dilutions were performed in an anaerobic workstation environment. Portions were spread-plated onto non-selective Blood Agar medium and Brain Heart Infusion (BHI) agar supplemented with hemin and vitamin K. Aerobic culture plates were incubated at 37°C for 48 hours, while anaerobic sets were grown inside gas-pack jars for 72–96 hours. Viable absolute bacterial load was recorded as Colony Forming Units per milligram of plaque sample (CFU/mg) [5].

Antibiotic Hypersensitivity Testing

Standardized bacterial suspensions corresponding to a 0.5 McFarland standard were uniformly distributed across Mueller-Hinton agar plates supplemented with 5% sheep blood for fastidious anaerobic isolates. Antibiotic hypersensitivity profiling was evaluated using the Kirby-

Bauer disc diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The chosen panel comprised amoxicillin ($10 \mu\text{g}$), metronidazole ($5 \mu\text{g}$), clindamycin ($2 \mu\text{g}$), and doxycycline ($30 \mu\text{g}$). Zone sizes (in millimetres) indicating clear pathogen growth inhibition were measured manually following overnight incubation periods.

Statistical Analytics

Bacterial CFU log transformations were compared across study tiers using the Kruskal-Wallis non-parametric test followed by post-hoc Dunn's validation, using SPSS 27 software. Mean zone diameters representing antibiotic susceptibility levels were analyzed using one-way ANOVA protocols. Significance boundaries were fixed at $p < 0.05$ using statistical tools in R v3.5.1 environments.

Results

Demographic and Clinical Phenotypes

Demographic criteria demonstrated distribution across groups. The average age was 34 ($\text{SD}=5$), and half of the participants were females (50%). The T2DM and comorbid T2DM_P groups showed significantly higher mean BMI indexes and elevated systemic markers compared to isolated periodontitis and healthy subjects ($p < 0.01$). Periodontal markers such as Probing Pocket Depth (PPD) and Bleeding on Probing (BOP) reached maximum values inside the comorbid T2DM_P tier.

3.2. Quantification of Plaque Bacterial Load

Absolute counts showed massive variance depending on patient health designation. Total culturable anaerobic counts expanded drastically inside the comorbid tier. Table 1 tracks quantified total culturable aerobic and anaerobic log counts across the four study divisions.

Table 1 Aerobic and Anaerobic Bacterial Loads Across Cohorts (Mean $\pm$ SD)

Patient Category	Sample Size (n)	Aerobic Load (log10 CFU/mg)	Anaerobic Load (log10 CFU/mg)
Healthy Controls (H)	10	4.65 ± 0.31	4.12 ± 0.28
Periodontitis (P)	10	5.12 ± 0.38	6.89 ± 0.51
Type 2 Diabetes (T2DM)	20	5.84 ± 0.44	5.21 ± 0.39
T2DM + Periodontitis (T2DM_P)	20	6.22 ± 0.49	7.84 ± 0.42

Antibiotic Hypersensitivity and Zone Profiles

Kirby-Bauer testing showed significant differences in drug susceptibility profiles across clinical states. Isolates from the T2DM_P group displayed reduced zones of inhibition to metronidazole and amoxicillin, indicating higher

resistance profiles. Doxycycline and clindamycin maintained wider inhibition parameters, showing strong efficacy profiles across cohorts. Table 2 presents the calculated mean zone sizes obtained from dental plaque bacterial isolates.

Table 2. Mean Zones of Inhibition (mm) Formed by Plaque Isolates

Antibiotic Disk	Healthy (H) (mm)	Periodontitis (P) (mm)	T2DM Only (mm)	T2DM_P Group (mm)
Amoxicillin ($10 \mu\text{g}$)	24.1 ± 1.5	16.4 ± 1.8	21.3 ± 1.4	14.2 ± 1.9
Metronidazole ($5 \mu\text{g}$)	22.8 ± 1.9	13.2 ± 2.1	19.5 ± 1.6	11.1 ± 2.4



Clindamycin (2 µg)	26.4 ± 1.2	21.8 ± 1.5	24.2 ± 1.1	19.8 ± 1.7
Doxycycline (30 µg)	25.9 ± 1.4	22.3 ± 1.7	23.8 ± 1.3	20.5 ± 1.6

Discussion

Our direct cultivation assessment reveals that metabolic deviations in Type 2 Diabetes Mellitus dramatically interact with periodontal inflammation to reshape plaque microbial dynamics. The prominent expansion of anaerobic bacterial load tracked inside the T2DM_P segment highlights how high-glucose microenvironments provide rich substrates for volatile proteolytic pathogens [12]. Hyperglycemia yields altered crevicular fluid compositions, serving as selective growth media for highly proliferative anaerobes [13].

The antibiotic hypersensitivity trends highlighted via Kirby-Bauer screening indicate notable patterns across Libyan clinical sectors. Isolates from comorbid patients displayed significantly smaller zones of inhibition for amoxicillin and metronidazole. This shift suggests increased antibiotic tolerance or resistance within these dense biofilm networks, potentially driven by accelerated horizontal gene transfer or frequent prior empiric drug exposure [6,14].

In contrast, clindamycin and doxycycline retained wider zones of inhibition across all cohorts. Doxycycline

provides a dual benefit due to its non-antibiotic matrix metalloproteinase (MMP) inhibition capabilities, directly mitigating local soft-tissue breakdown [7]. These findings emphasize that routine screening of culturable bacterial loads and antibiotic hypersensitivity should inform therapeutic choices, avoiding excessive reliance on empirical amoxicillin regimens.

Conclusions

In conclusion, early-onset Type 2 Diabetes Mellitus combined with periodontitis severely intensifies viable plaque bacterial loads while significantly altering antibiotic hypersensitivity boundaries. Culturable testing provides an accessible, cost-effective method to optimise dental antibiotic choices and support targeted, integrated medical-dental treatment paths in resource-constrained regions.

Conflict of Interest

The authors declare that they have no conflict of interest

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