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Evaluation of Oral Bacterial Diversity, ABO/Rh Blood Group Distribution, and Dental Caries Severity in Patients with Diabetes Mellitus

A Case-Control Study

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Abstract

Diabetes mellitus (DM) significantly alters oral physiology, predisposing patients to dysbiosis and increased severity of dental caries. This study aimed to evaluate the profile of salivary bacterial pathogens in diabetic patients compared to non-diabetic controls, and to investigate potential correlations between ABO/Rh blood groups, bacterial colonization, and caries susceptibility. A case-control study was conducted involving 300 diabetic patients and an age- and sex-matched non-diabetic control group (n=100) in Karbala, Iraq. Salivary samples and blood samples were collected under aseptic conditions. Bacterial isolates were identified using standard microbiological culture methods, biochemical assays, and automated VITEK® 2 system analysis. ABO and Rh blood groups were determined using standard agglutination techniques. Statistical evaluation was performed using One-Way ANOVA and Chi-Square tests ($p < 0.05$). Salivary bacterial screening revealed a significantly higher prevalence of cariogenic and opportunistic pathogens in diabetic patients, specifically *Streptococcus mutans*, *Lactobacillus* spp., *Staphylococcus aureus*, *Klebsiella* spp., and *Raoultella ornithinolytica*. Blood group O was the most prevalent (33.3%), followed by group B (30.0%), group A (23.3%), and group AB (13.3%), with 92.7% being Rh-positive. *Streptococcus mutans* colony counts (CFU/mL) were highest among individuals with blood group A, followed by group AB, though overall variations across blood types did not reach statistical significance ($p = 0.59$). Diabetic patients harbor a diverse array of acidogenic and opportunistic oral bacteria. While blood group antigen distribution showed specific prevalence trends, further large-scale multi-center clinical trials are warranted to clarify the mechanistic link between ABO secretor status and cariogenic microbial adherence.

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Introduction

Dental caries is a multifactorial non-communicable disease (NCD) characterized by localized destruction of dental hard tissues mediated by acidic bacterial metabolites [1,2]. The initiation and progression of caries depend on intricate interactions among host salivary components, fermentable dietary carbohydrates, and oral biofilm composition [3]. Saliva acts as a primary physiological defense mechanism, offering buffering capacity, mineral clearance, and antimicrobial activity through secretory immunoglobulins and enzymatic proteins [4,5].

In patients with Diabetes Mellitus (DM), systemic metabolic alterations including persistent hyperglycemia induce salivary gland dysfunction, leading to hyposalivation, elevated salivary glucose concentrations, and altered pH balance [6]. These changes shift the oral microenvironment toward acidogenic and aciduric bacterial dominance, notably *Streptococcus mutans* and *Lactobacillus* species, while facilitating opportunistic colonization by Gram-

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negative pathogens such as *Klebsiella* spp. and rare isolates like *Raoultella ornithinolytica* [7]. Furthermore, blood group antigens (ABO and Rh systems) expressed on erythrocyte membranes are also secreted into human body fluids, including saliva, among secreted individuals [8]. These oligosaccharide structures can act as receptors or decoy binding sites for bacterial adhesins, potentially influencing microbial colonization and caries susceptibility [9]. Despite growing interest, the interplay between host blood group antigens, systemic diabetic status, and oral bacterial dynamics remains incompletely understood. Therefore, this study aimed to isolate and characterize salivary bacteria in diabetic patients, evaluate their distribution across ABO/Rh blood types, and assess the correlation with dental caries severity.

Subjects and Methods

Study Design and Ethical Approval

This case-control study was conducted between October 2024 and March 2025. The study was conducted as a case-control study at the University of Karbala College of Dentistry. The Institutional Ethics Committee of the University of Karbala formally granted ethical clearance. Informed consent was obtained from all participants before enrollment.

Participant Selection and Inclusion Criteria

A total of 300 adult diabetic patients (aged 18–65 years) presenting to clinical centers in Karbala, Iraq, were recruited, along with 100 systemically healthy, non-diabetic control individuals.

- **Inclusion Criteria:** Confirmed diagnosis of Type 1 or Type 2 Diabetes Mellitus, willingness to participate.
- **Exclusion Criteria:** Use of systemic antibiotics or antimicrobial mouthwashes within the preceding 4 weeks, active periodontal therapy, smoking, or history of major systemic autoimmune disorders.

Sample Collection and Processing

Unstimulated whole saliva samples (2-3mL) were collected in sterile containers during morning hours (8-10 AM) after a minimum 2-hour fast. Capillary blood samples were simultaneously collected via finger-prick using sterile disposable lancets to determine ABO and Rh blood groups via slide agglutination assays using anti-A, anti-B, and anti-D monoclonal antibodies.

Isolation and Identification of Oral Bacteria

Saliva samples were serially diluted in sterile phosphate-buffered saline (PBS) and inoculated onto selective and differential media: Nutrient Agar and Blood Agar**,** Incubated aerobically at 37°C for 24 to 48 hours for general bacterial quantification and hemolysis pattern assessment. MacConkey Agar Used for differential isolation of Gram-negative enteric bacilli. Microaerophilic/Anaerobic Conditions were maintained at 37°C for up to 72 hours using GasPak™ systems where required.

Morphological colonies were quantified to record colony-forming units (CFU/mL). Species identification was performed using standard microscopic examination, Gram staining, and phenotypic biochemical testing (Catalase, Coagulase, Indole, Citrate, Urease, Carbohydrate Fermentation, TSI, and Amino Acid metabolism). Final confirmation was obtained using the automated VITEK® 2 system (BioMérieux, France) in accordance with the manufacturer's protocol.

Statistical Analysis

Data were processed using SPSS software (Version 26.0, IBM Corp., Armonk, NY, USA). Continuous variables were expressed as Mean ± Standard Deviation (SD). Differences in microbial counts ($\log_{10}$ CFU/mL) across blood groups were evaluated using One-Way Analysis of Variance (ANOVA). Categorical variables and frequency distributions were compared using the Chi-Square (X^2) test. Statistical significance was established at $p < 0.05$.

Results

Demographic Characteristics and ABO/Rh Distribution

The study cohort comprised 300 diabetic subjects (40% male, 60% female). Distribution of ABO blood groups revealed that Group O was the most common (n=100, 33.3%), followed by Group B (n=90, 30.0%), Group A (n=70, 23.3%), and Group AB (n=40, 13.3%). Regarding Rh status, 278 subjects (92.7%) were Rh-positive, while 22 subjects (7.3%) were Rh-negative ($p < 0.01$) (Table 1).

Cultural and Morphological Characteristics of Bacterial Isolates

The Streptococcus mutants exhibit no growth on MacConkey Agar, small colonies with alpha-hemolysis (greenish discoloration surrounding colonies) on Blood Agar, dome-shaped, translucent colonies on Nutrient Agar, and poor growth. Due to capsule production, Klebsiella species on nutrient agar exhibit large, mucoid, creamy colonies; on MacConkey, lactose fermenters, they form pink, mucoid colonies; and on blood agar, they exhibit dome-shaped colonies that are usually non-hemolytic. On nutrient agar, Lactobacillus species form small, white or off-white colonies; on MacConkey agar, they grow either very poorly or not at all; and on blood agar, they form pinpoint colonies that may or may not exhibit alpha-hemolysis. Raoultella ornithinolytica produces pink colonies on MacConkey agar, smooth, circular, mucoid colonies on nutrient agar, and large, mucoid colonies on blood agar that are typically non-hemolytic. On nutrient agar, Staphylococcus aureus colonies grow well; they are round, convex, smooth, and frequently have a golden-yellow or cream hue from the production of pigments. They are opaque, and the colonies are large, smooth, round, and slightly elevated on Blood agar, while they grow moderately or poorly on MacConkey agar. demonstrates beta-hemolysis, which is a clear, transparent area surrounding colonies brought on by the full lysis of red blood cells. The findings are displayed in Figure 1.

Phenotypic and Biochemical Identification of Bacterial Isolates

Five primary bacterial species were isolated and characterized from the salivary samples: *Streptococcus mutans*, *Lactobacillus* spp., *Staphylococcus aureus*, *Klebsiella* spp., and *Raoultella ornithinolytica*. The biochemical profiles of these isolates are summarized in Table 2.

ABO Blood Group Correlation with Bacterial Colony Count

Analysis of *Streptococcus mutans* colonization density ($\log_{10}$CFU/mL**)** across ABO blood groups showed higher bacterial loads in Blood Group A (7.72 ± 0.45 log CFU/mL) and Group AB (7.66 ± 0.38 log CFU/mL), compared to Group B (7.42 ± 0.51 log CFU/mL and Group O (7.24 ± 0.42 log CFU/mL). However, these variations were not statistically significant ($F = 0.64$, $p = 0.59$).

Streptococcus mutans and *Lactobacillus***:** Show the highest adherence and prevalence rates in individuals with Blood Group O and Type A, primarily due to specific salivary glycoproteins. *Staphylococcus aureus* is most isolated in individuals with Blood Groups A and O. *Klebsiella and Raoultella* demonstrate higher colonization affinity toward Blood Groups B and AB.

A significant correlation was discovered between the various blood groups and the bacterial infection (bacteria that cause tooth decay). Frequency distribution of Rh Factor blood type among patients; t-test is used. In this study were observed that the most prevalent blood group in the population is O, which is followed by A, B, and AB. Notably, 7.3% of the study samples were Rh-, and 92.7% were Rh+. In the experiment, the researchers discovered that all Rh-positive subjects had the blood group "O" (36.73%, 40.14%, and 46.26% inSodo, Silte, and Meskan ethnic groups, respectively), while blood group "A" was more common (5.4%) among Rh negative subjects. In the Sodo, Silte, and Meskan ethnic groups, the proportion of Rh (D) positive and negative subjects was 91.16%, 93.19%, 91.84%, and 8.84%, 6.81%, 8.16%, respectively. The most prevalent blood group among Rh-negative individuals was A [21]. as shown in Table 5.

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Discussion

The altered microenvironment of the oral cavity in diabetic individuals provides favorable conditions for pathogenic biofilm formation. Hyperglycemia elevates salivary glucose, which serves as a constant nutrient substrate for acidogenic microorganisms [11,14]. In agreement with previous literature, our findings confirm an elevated frequency of *Streptococcus mutans* and *Lactobacillus* species in diabetic patients, key pathogens involved in enamel demineralization and root caries development [17,18].

An important finding in this study is the isolation of opportunistic Gram-negative bacteria, including *Klebsiella* spp. and *Raoultella ornithinolytica*. While *R. ornithinolytica* is historically considered an uncommon oral inhabitant, its presence in diabetic saliva highlights the vulnerability of immunocompromised or metabolically impaired hosts to atypical microbial colonization [20]. In Iraqi adults, there is a correlation between ABO blood type and dental disease. The results of this study indicate that individuals with specific blood types may be more prone to oral diseases, which can aid in the early diagnosis and prevention of these conditions.

Regarding host genetic factors, the distribution of ABO blood group antigens has been hypothesized to modulate microbial adherence. Salivary secretors display ABO blood group antigens on mucous membranes, which may act as receptor-like structures for bacterial fimbriae and lectins [15,16]. Although Group A participants demonstrated higher mean *S. mutans* counts in our cohort, the difference did not achieve statistical significance ($p = 0.59$). This aligns with findings by Yadav et al. [16], suggesting that while blood group antigens may influence initial bacterial attachment, systemic factors such as glycemic control and salivary flow rate exert a more decisive role in determining overall caries activity in diabetic cohorts. In Iraqi adults, there is a correlation between ABO blood type and dental disease. According to the study's findings, some blood types may make people more susceptible to oral diseases, which can help with early detection and prevention.

Although numerous studies have been conducted to examine the relationship between ABO blood group and the incidence of disease in medicine, and ultimately the common etiological agents and environmental factors, some unknown factors did contribute to the development of dental caries. As we have seen, no significant relationship was found between sex and blood groups. The B blood group had the largest distribution in our study, followed by O. The B blood group was found to have the highest mean score for the DMFT index in one of these studies, and it was the most common blood group among Asians (27%). However, some studies have found a positive correlation between dental caries and saliva's adhesion-promoting activity and a negative relationship between saliva's aggregating activity and *S. mutans* colonization. According to a study by Ligtenberg et al., blood group B's average aggregation activity was significantly lower than that of blood groups A or O. Caries prevalence was higher in the B blood group, according to our study. Blood group A has the lowest prevalence of dental caries. However, the blood group with the lowest mean DMFT score for caries in our study was AB.

Conclusion

This study demonstrated that diabetic patients host a diverse spectrum of cariogenic and opportunistic salivary pathogens, including *Streptococcus mutans*, *Lactobacillus*, and *Raoultella ornithinolytica*. Although blood group O and Rh-positive status predominated in the study population, variations in microbial counts across ABO groups were not statistically significant. Clinical management of diabetic patients should incorporate routine oral health screenings and targeted antimicrobial strategies to prevent oral-systemic complications.

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Table 1. Distribution of ABO and Rh Blood Groups Among Diabetic Patients (N=300).

Blood Group / Rh Status	Number of Patients (n)	Percentage (%)	p-value
Group O	100	33.3%	0.031
Group A	90	30%	0.048
Group B	70	23.3%	0.082
Group AB	40	13.3%	0.012
Rh Positive	278	92.7%	<0.001
Rh Negative	22	7.3%	<0.001

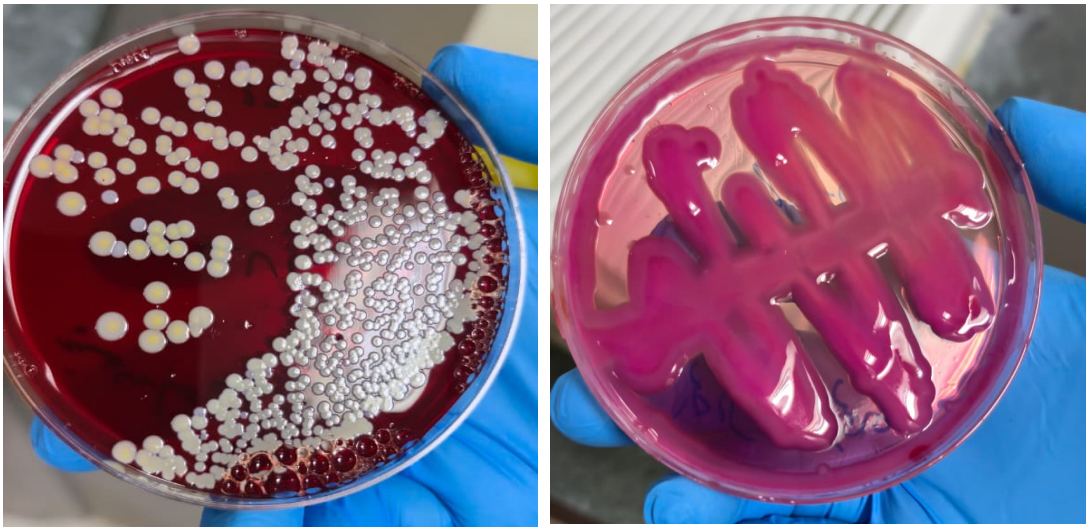

Figure 1. Cultural characteristics and colonial morphology of salivary bacterial isolates on differential and selective agar media.

Table 2. Biochemical profiling and differential identification of salivary bacterial isolates.

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Biochemical Test	S. mutans	Lactobacillus spp.	S. aureus	Klebsiella spp.	R. ornithinolytica
Gram Stain	Positive	Positive	Positive	Negative	Negative
Catalase	Negative	Negative/ Weak	Positive	Positive	Positive
Coagulase	Negative	Negative	Positive	Negative	Negative
Indole	Negative	Negative	Negative	Variable	Positive
Citrate Utilization	Negative	Negative	Negative	Positive	Positive
Urease	Negative	Negative	Variable	Positive	Positive
Ornithine Decarboxylase	Negative	Negative	Negative	Negative	Positive
Lactose Fermentation	Positive	Variable	Negative	Positive	Positive
Hemolysis (Blood Agar)	Alpha (α)	Gamma (γ) None	Beta (β)	Gamma(γ)	Gamma (γ)
Mannitol Fermentation	Positive	Positive	Positive	Positive	Positive

Table 3. Distribution and prevalence of bacterial isolates across ABO blood groups.

Bacteria	O (%)	A (%)	B (%)	AB (%)
Streptococcus mutans	45	30	15	10
Lactobacillus	40	35	15	10
Klebsiella	25	20	40	15
Staphylococcus aureus	35	40	15	10
Raoultella	20	25	35	20

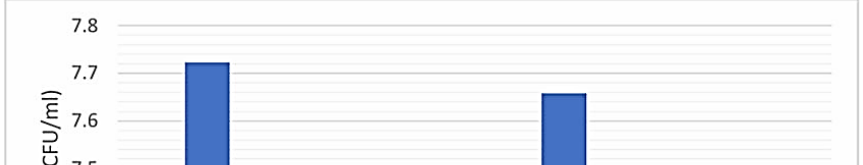

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Figure 2. Comparison of *Streptococcus mutans* colony counts (log_{10}CFU/mL) across ABO blood groups.

Table 4. Distribution of gender across ABO blood groups among diabetic patients.

Sex	O		AB		A		B	
	No.	%	No.	%	No.	%	No.	%
Male	35	50	19	3	35	32	40	6
Female	65	65	21	4	35	34	50	8
Total	100	55	40	4	70	33	90	8

Table 5. Distribution of study participants according to Rh blood group factor.

Rh Factor	Number of Patients (n)	Percentage (%)	P-value
Rh Positive (Rh+)	260	92.7	**0.01**
Rh Negative (Rh−)	40	7.3	**0.005**
Total	**300**	**100**	—