Original Article

Isolation, Identification and Phenotypic Analysis of the Oral Bacterial Species from Supragingival Dental Plaque and Their Association with Different Factors

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ABSTRACT

Objective

The objective of this study was to isolate and characterize oral aerobic, facultative anaerobic, and microaerophilic bacterial species from supragingival plaque and to evaluate the association of different demographic, phenotypic, and biochemical characteristics with the identified organisms.

Material and Methods

This prospective cross-sectional study enrolled 101 bacterial isolates sampled from the supragingival plaque, using sterile swab. Oral bacterial species were identified through biochemical and phenotypic analysis using standardized methods. The inclusion criteria were participants of Karachi, residents of both genders, being at least eighteen years old, having a Loe and Silness plaque index. Participants with severe systemic illnesses, such as immunodeficiency disorders, on chemotherapeutic drugs, antibiotic therapy twelve weeks before sampling persistent systemic infections, or known communicable diseases, were excluded. All details were recorded on proforma and data was analyzed by using SPSS version 22.0. p-value of < 0.05 was considered significant.

Results and Discussion

Multiple bacterial species were identified, with Gram-positive species predominating. Statistically significant variables included gender, age, occupation, hemolysis on blood agar, bile esculin hydrolysis, catalase, oxidase, ONPG, CIT, MALO, ODC, UREA, VP, GEL, nitrate, sucrose, mannitol, melibiose, and raffinose. It was found that considerable variation in the oral bacterial species exists influenced by multiple factors.

Conclusion

It has been concluded that multiple and variable Gram-positive and negative bacterial species have been isolated from supragingival dental plaque of local isolates. The demographic, phenotypic, and biochemical characteristics demonstrated statistically significant associations with the identified organisms.

Keywords

Biofilm; Oral Health; Oral Bacterial Species; Supragingival Plaque

INTRODUCTION

An essential component of preserving oral health is the oral microbiota, a dynamic and varied microbial population found in the human oral cavity [1]. Notably, the oral cavity has been found to have over 700 different types of bacteria, making it the second most varied microbiota after the gut [2]. The most prevalent oral bacterial

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genera include *Staphylococcus* spp., *Streptococcus* spp., *Granulicatella* spp., *Gamella* spp., *Neisseria* spp., *Bacteroides* spp. *Actinomyces* spp. *Treponema* spp., *and Veillonella* spp.[3] They are essential for immune system control, colonization resistance, digestion, and other processes[4].

Oral bacteria and the host's immune system together maintain immunological homeostasis and dental health [5]. However, oral conditions including periodontitis, gingivitis, and dental caries can be exacerbated by dysbiosis, a disorder in which the equilibrium of microorganisms is disturbed. This imbalance is influenced by several factors, such as lifestyle habits, medical problems, smoking, alcohol consumption, and food [6].

Oral microbiota can reach the circulation through the periodontal pockets or oral mucous membrane, alter host defense, and produce inflammatory mediators that can cause disease at many body locations. It can result in respiratory diseases such as pneumonia, osteomyelitis, diabetes, cancer, and bacterial endocarditis [7].

These factors lead to multiple dental diseases which may affect an individual's personality but also have a significant negative impact on a nation's economy [8]. The anticipated global economic impact of oral problems in 2019 was estimated to be $710 billion, of which $387 billion was directly spent on treatment. While this figure includes several oral illnesses, dental caries is responsible for a significant amount of these expenses[9]. The World Health Organization (WHO) estimates that over 1 billion people worldwide suffer from serious periodontal (gum) diseases [10].

The microbiological composition of dental plaque is strongly associated with a number of systemic and oral disorders. Dental caries, periodontal illnesses, and systemic conditions including diabetes and cardiovascular disease are all significantly influenced by dental plaque, a biofilm made up of many microorganisms. [11]. Furthermore, a higher risk of atherosclerosis and poor glycemic control in diabetics have been associated with dysbiosis in the plaque microbiota [12]. Therefore, the microbiological composition of plaque has considerable effects on oral and general health.

Microbial diversity is determined by combination of regional factors, including nutrition, water quality, socioeconomic status, habits, and cultural customs, which may contribute to different patterns of dental diseases. Furthermore, examining differences in the local microbiota can help with early illness detection and enhance community oral health outcomes. The urgent need for an in-depth investigation of the bacterial organization in supragingival dental biofilm caries and the most common bacterial species in the local population is immediately addressed by this research. Using culture-based and biochemical identification, it also aims to close the knowledge gaps off local populace regarding the composition of the oral microbiome and other parameters. Since there is a dearth of information on the subject matter in the Karachi local population, by advancing our knowledge of supragingival plaque microbiologically, this research provides insightful information. A comprehensive profile of the microbial diversity in the oral cavity can be obtained by isolating and describing aerobic, facultative anaerobic, and microaerophilic bacterial species. Finding correlations between bacterial species and biochemical, phenotypic, and demographic traits can reveal microbial ecology, colonization patterns, and possible dysbiosis risk factors. In addition to encouraging early diagnosis techniques, preventive measures, and the creation of focused oral hygiene programs, these findings advance the field by creating a fundamental database for upcoming clinical or epidemiological investigations.

MATERIALS AND METHOD

Study design and duration

This prospective experimental study was conducted at the Department of Microbiology, Jinnah University for Women, with study duration of one year from September 2023 and September 2024. Study recruits 101 bacterial isolates through non-probability, consecutive sampling technique.

Sample size

The sample size of 101 bacterial isolates was calculated through an online calculator with a confidence interval of 95%, margin of error of 5%, and population proportion of 50%.

DATA COLLECTION

Inclusion and Exclusion Criteria

Inclusion criteria was participants of Karachi residents of both genders, >18 years, having a Loe and Silness plaque index of one to three[13]. This index incorporates

0 means No plaque in the gingival area, 1 means film of plaque adhering to the free gingival margin and adjacent area of the tooth. Plaque is visible only after using a disclosing solution or with careful examination, 2 means moderate accumulation of plaque along the gingival margin. Plaque is visible to the naked eye. 3 means heavy accumulation of plaque at the gingival margin and interdental spaces. Thick deposits are clearly visible. Participants with severe systemic illnesses, such as immunodeficiency disorders, on chemotherapeutic drugs, persistent systemic infections, or known communicable diseases and using antibiotic therapy twelve weeks before sampling were excluded.

Phenotypic Analysis

After taking informed verbal consent the demographic details were recorded on a predesigned proforma. Sterile swabs were used to collect microbial samples from plaque. All samples were transferred to the lab for testing while suspended in nutrient broth and kept in frozen containers which was incubated at 37 °C. They were streaked on blood and nutrient agar for the assessment of their morphological characteristics and hemolytic pattern analysis. The isolated colonies were Gram-stained and examined under microscope. Lactose fermentation, bile esculin tests and catalase test were performed on McConkey agar, bile esculin agar and hydrogen peroxide respectively.

Biochemical Analysis

Gram-positive bacteria were identified by using biochemical strips which were locally formulated in the laboratory of the Department of Microbiology, Jinnah University for Women. Gram-negative bacilli were identified using QTS (DESTO BRC) [14] (Figure-1a, b). For multiple isolates of same bacterial spp. were assessed using criteria developed by guidelines of api strept (bioMérieux, api 20 strep (20 600, 07625L - en - 2010/07),), api staph (bioMérieux, api Staph. (20 500, 07468L - en - 2013/09) and Cowen steel manual [15]. The bacterial spp. having >70 percent positive results has been considered as positive, test results between 50-70 has been considered as differential (d) and < 50 has been considered as negative for the respective biochemical determinants. All the observations have been recorded on the proforma and specific bacterial species has been tracked following an identification scheme designed by Cowan and Steel's manual for the identification of medical bacteria.

Statistical analysis

The data was entered and analyzed using SPSS v 22.0. Descriptive statistics, including mean, standard deviation, and frequency, were used to examine the participants' demographic data, Frequencies and percentages were used to report the Plaque index. The association between qualitative factors and bacterial morphology was observed using the chi-square test. The p-value of < 0.05 was considered as significant.

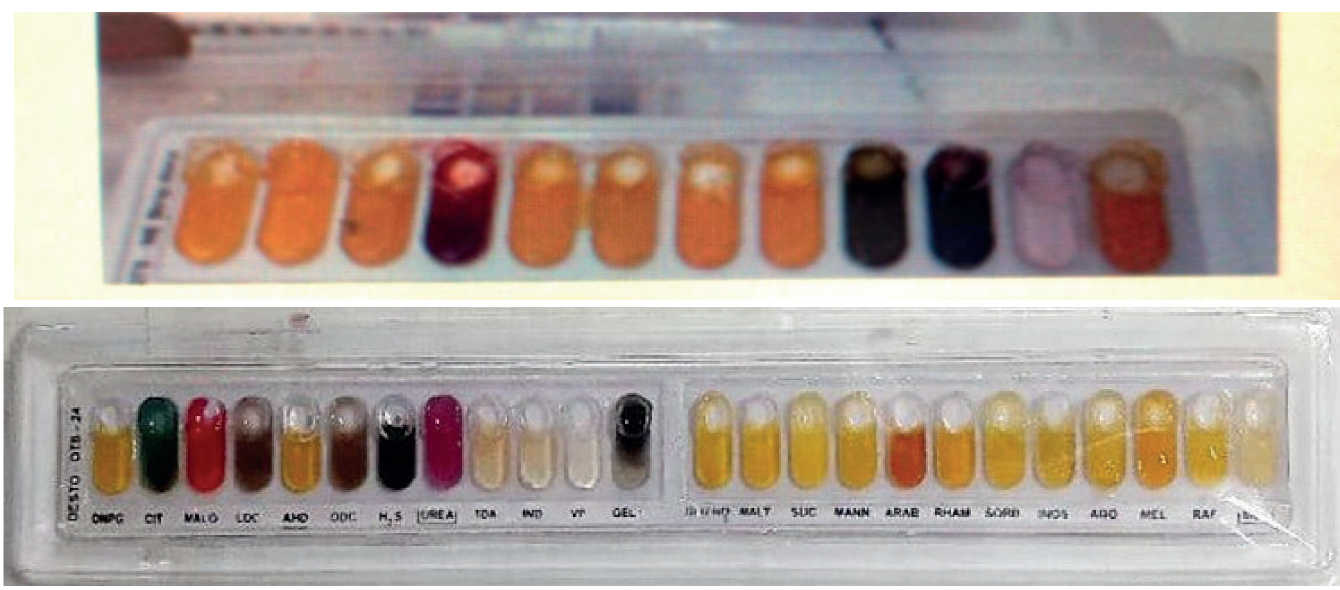

Fig 1a, b: Quick Testing Strip (QTS) for biochemical identification of Gram positive (a) Gram negative (b) bacterial isolates.

ETHICAL CLEARENCE

Ethical review letter was taken from ethical review board of Jinnah University for women no: JUW/IERB/SCI-ARA-003/2025.

RESULTS

Demographic variables analysis

The mean age of the participants was 34.39 ± 11.87 years, with a range of 18 to 64 years. The 37.6 %(n = 28) of respondents were between the ages of 20-30 years, followed by 40-50 years i.e. 27.7 (28), then 30-40 years 19.8%(20). The majority of responders (71.3%) were males. Furthermore, 80.2% (n=81) of participants were middle class, followed by low 15.8 (16) and high class 4.0 (4). Among participants 25.7% (26) were entrepreneurs, followed by 22.8 (23) had private jobs and students. And the majority of participants (97%), spoke Urdu. Approximately 26.7 % (27) had mild plaque 43.6% (n=44) had moderate plaque, while 29.7 % (30) had severe plaque.

Phenotypic analysis

The isolation of oral bacterial species revealed that overall Gram-positive bacteria accounted for 92.1% (n=93) of the organisms, while Gram-negative bacteria formulate 7.9% (n=8). Among these, cocci constituted 50.5% (n=51), whereas bacilli accounted for 48.5% (n=49) and 1 coccobacillus. Among the cocci, Gram-positive cocci account for 94.11% (n=48), followed by Gram-negative cocci at 5.88 % (n=3), and Gram-negative coccobacilli at 1.9 % (n=1). Among the bacillus, Gram-positive bacilli accounted for 91.3% (n=45) while Gram-negative bacilli accounted for 8.13% (n=4). Regarding hemolysis patterns, some microorganisms may show different types of hemolysis under different conditions. The frequency of beta, alpha, and gamma hemolysis was almost equal among the bacterial species i.e. 34.7% (n=35), 32.7% (n=33), and 28.7% (n=29) respectively. However, some microorganisms showed variable hemolytic patterns like Alpha/Beta hemolysis was detected in 3.0% (n=3) of cases, while 1 % (1) of organisms showed gamma/alpha hemolysis. The majority of microorganisms, 68.3% (n=69), tested positive for the bile esculin test, while 26.7% (n=27) were negative, and 5.0% (n=5) showed variable results. Similarly, most microorganisms were non-lactose fermenters i.e. 80.2% (n=81), followed by lactose-fermenting microbes and differential i.e. mixed non-lactose and lactose fermenters organisms 9.9% (n=10) each.

Biochemical analysis

The biochemical analysis includes sugar fermentation and enzymatic degradation by oral bacteria using custom-made strips for Gram-positive and Gram-negative bacteria (Tables 1-4). All organisms showed variable results of biochemical tests. A total of 29 bacterial species have been isolated belonging to three different phyla (Figure- 2). Table- 5 shows the percentage distribution and taxonomical classification of oral bacterial isolate. Majority bacteria behave as pathogens or maintain a commensal /pathogen relationship. The variables that demonstrate a significant statistical association with isolated oral bacterial species were gender, age, occupation, hemolysis on blood agar, bile esculin hydrolysis, catalase, oxidase, ONPG, CIT, MALO, ODC, UREA, VP, GEL, Nitrate, Sucrose, Mannitol, Melibiose, and Raffinose (Table-6).

Table 1a. The biochemical characteristics of Gram positive cocci oral bacterial isolates

	Staph. aureus (n=8)	Staph. epidermidis (n=7)	Staph. caprae (n=4)	Strept. mitis (n=2)	Strept. angiosus (n=1)	Strept. bovis (n=1)	Strept. canis (n=1)	Strept. intermedius (n=3)
Microscopy	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	+
Catalase	+	-	-	d*	-	-	-	-
Sucrose	+	+	-	+	+	+	+	+
Raffinose	-	-	+	-	+	-	-	-
Mannitol	+	-	d*	-	+	-	-	-
Glucose	+	+	d*	d*	+	+	+	+

	Staph. aureus (n=8)	Staph. epidermidis (n=7)	Staph. caprae (n=4)	Strept. mitis (n=2)	Strept. angiosus (n=1)	Strept. bovis (n=1)	Strept. canis (n=1)	Strept. intermedius (n=3)
Lactose	+	+	+	+	+	d*	+	+
Sorbitol	-	-	+	-	+	-	-	+
Maltose	+	+	+	d*	+	d*	+	-
Arginine	-	-	-	-	-	-	+	-
TDA	+	d*	+	+	+	+	+	+
IND	-	-	-	-	-	-	-	-
VP	+	-	-	-	+	-	-	-
GEL	+	+	-	+	+	+	+	+
Urea	+	-	-	d*	-	-	-	-
Nitrate	+	+	+	d*	+	+	+	-

*d = differential result

Table 1b. The biochemical characteristics of Gram positive cocci oral bacterial isolates

	Allaicoccus otitis (n=2)	Enterococcus feacalis (n=5)	Entercococcus feacium (n=1)	Rothia mucilaginosa (n=8)	Micrococcus luteus (n=5)
Microscopy	+	+	+	+	+
Oxidase	-	-	-	-	+
Catalase	+	-	-	d*	+
Sucrose	-	+	+	+	-
Raffinose	-	-	-	-	-
Mannitol	-	+	+	+	-
Glucose	d	+	+	+	-
Lactose	+	+	+	+	+
Sorbitol	-	+	+	+	-
Maltose	-	+	+	+	-
Arginine	-	-	-	-	-
TDA	d	+	+	+	-
IND	-	-	-	-	-
VP	-	-	+	+	-
GEL	d	+	+	+	-
Urea	-	-	+	-	-
Nitrate	d	+	+	+	+

*d = differential result

Table 2. The biochemical characteristics of Gram negative cocci oral bacterial isolates

	Neissaria mucosa (n=1)	Gamella haemolysans (n=2)
Microscopy	+	+
Oxidase	+	-
Catalase	+	+
Sucrose	d*	+
Raffinose	+	+
Mannitol	+	+
Glucose	+	+
Lactose	+	d*
Sorbitol	+	+
Maltose	+	+
Arginine	-	-
TDA	+	+
IND	-	d*
VP	+	+
GEL	+	+
Urea	-	d*
Nitrate	+	+

*d = differential result

Table 3. showing biochemical characteristics of Gram positive bacilli oral bacterial isolates

	Corynebacterium matruchotii (n=6)	Lactobacillus Salivarious (n=7)	Lactobacillus casei (n=12)	Bacillus coagulans (n=1)	Bacillus megaterium (n=3)	Bacillus licheniformis (n=1)	Actinomyces odontolyticus (n=4)	Actinomyces viscosus (n=1)	Rothia dentocariosa (n=9)
Gram Reaction	+	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	-	-	-
Catalase	d*	+	+	+	+	d*	d*	+	d*
ONPG	+	+	+	+	+	+	+	-	+
CIT	-	+	+	+	+	+	d*	+	-
URE	-	-	-	-	-	-	-	-	-

	Corynebacterium matruchotii (n=6)	Lactobacillus Salivarious (n=7)	Lactobacillus casei (n=12)	Bacillus coagulans (n=1)	Bacillus megaterium (n=3)	Bacillus licheniformis (n=1)	Actinomyces odontolyticus (n=4)	Actinomyces viscosus (n=1)	Rothia dentocariosa (n=9)
VP	-	+	+	+	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+
Nitrate	-	-	+	-	d*	-	-	-	-
Maltose	-	+	+	-	+	-	+	-	+
Mannitol	-	+	+	-	-	-	+	+	+
Arabinose	-	+	+	+	+	+	-	-	-
Melibiose	-	+	+	+	-	+	-	-	-
Raffinose	-	+	+	+	-	+	-	-	-
Cytochrome Oxidase	-	+	+	-	-	+	+	+	+

*d = differential result

Table 4. showing biochemical characteristics of Gram negative bacilli oral bacterial isolates

	Acinetobacter iwoffi (n=2)	Acinotobacter calcoaceticus (n=1)	Citrobacter fruendii (n=1)	Enterobacter gergovea (n=1)
Motility	-	-	-	-
Catalase	-	-	-	-
ONPG	-	-	+	+
CIT	-	-	+	+
MALO	-	-	-	+
LDC	-	-	-	+
ADH	-	+	-	-
ODC	-	+	-	+
H2S	-	-	-	-
URE	+	-	-	+
TDA	+	+	+	+
IND	-	-	-	-
VP	-	+	-	+
GEL	-	-	-	-

	Acinetobacter iwoffi (n=2)	Acinotobacter calcoaceticus (n=1)	Citrobacter fruendii (n=1)	Enterobacter gergovea (n=1)
Glucose	+	+	+	+
Nitrate	+	-	+	-
Maltose	+	-	-	+
Sucrose	+	+	-	+
Mannitol	-	-	+	+
Arabinose	-	-	+	+
Rhamnose	-	-	+	+
Sorbitol	-	-	+	+
Inositol	-	-	-	+
Adonitol	-	-	-	+
Melibiose	-	-	+	+
Raffinose	-	-	+	+
Cytochrome Oxidase	-	-	-	-

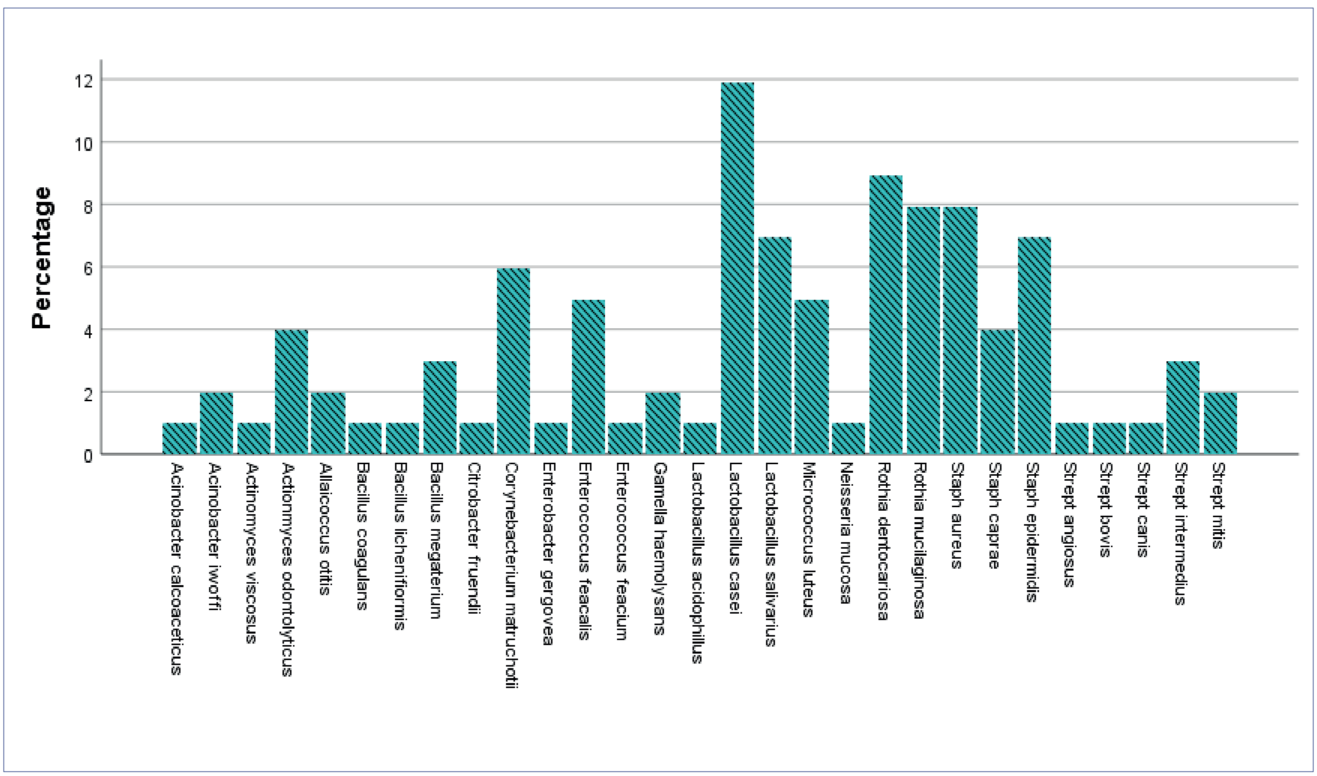

Fig.2: shows percentage distribution of identified oral bacterial species.

Table 5. showing percentage distribution and taxonomical classification of oral bacterial isolates

Phylum	Genus	Specie	%
Firmicutes	*Staphylococcus*	*aureus* *epidermidis* *caprae*	7.9 6.9 4.0
	Streptococcus	*mitis* *angiosus* *bovis* *canis* *intermedius*	2.0 1.0 1.0 1.0 3.0
	Alloicoccus	*otitis*	2.0
	Enterococcus	*feacalis* *feacium*	5.0 1.0
	Lactobacillus	*Salivarious* *casei*	6.9 11.9
	Bacillus	*coagulans* *megaterium* *licheniformis*	1.0 3.0 1.0
	Gamella	*haemolysans*	2.0
	Micrococcus	*luteus*	5.0
Actinobacteria	*Corynebacterium*	*matruchotii*	5.9
	Rothia	*mucilaginosa* *dentocariosa*	7.9 8.9
	Actinomyces	*odontolyticus* *viscosus*	4.0 1.0
Proteobacteria	*Acinetobacter*	*iwoffi* *calcoaceticus*	2.0 1.0
	Enterobacter	*gergovea*	1.0
	Citrobacter	*fruendii*	1.0
	Neisseria	*mucosa*	1.0

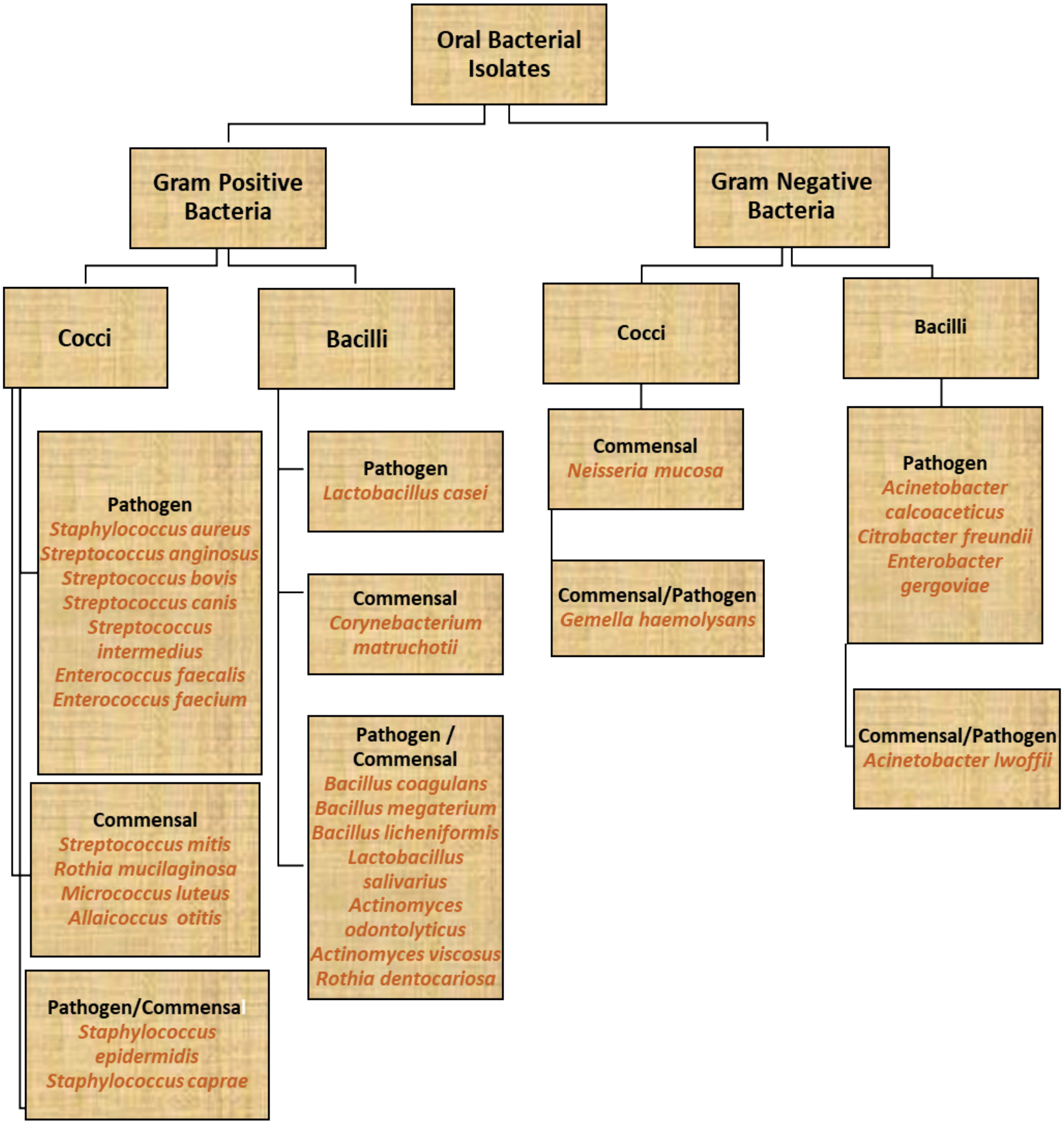

Fig -3: Flow chart demonstrating distribution of oral isolates into different taxonomic classification

Table 6: Association between variables with organism identified

Variable	p-value
Gender	0.012*
Age	0.007*
Occupation	0.000*
Plaque index	0.624
Hemolysis on blood agar	0.000*
Lactose fermentation	0.126
Bile esculin hydrolysis	0.040*
Catalase	0.030*
Oxidase	0.000*
ONPG	0.039*
CIT	0.027*
MALO	0.010*
LDC	0.075
ADH	0.124
ODC	0.011*
H2S	0.974
UREA	0.002*
TDA	0.124
IND	0.463
VP	0.000*
GEL	0.023*
Glucose	0.521
Nitrate	0.033*
Maltose	0.740
Sucrose	0.000*
Mannitol	0.000*
Arabinose	0.068
Rhamnose	0.691
Sorbitol	0.072
Inositol	0.898
Adonitol	0.608
Melibiose	0.011*
Raffinose	0.001*

*significant p-value< 0.05

DISCUSSION

In this prospective, cross-sectional study the determined mean age was 34.39 ± 11.87 years. Age-related variations in plaque composition reflect variations in host physiology, immunological response, and dental care practices. Early colonizers like Streptococcus and *Actinomyces* species, which are typically linked to oral health, compose the majority of plaque in younger people. On the contrary, microbial diversity is generally greater in older adults, with a greater number of pathogenic species such as *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola,* particularly when periodontal diseases are present [16]. The significance of considering age into account as a demographic element in oral microbiological research is highlighted by these age-associated modifications in plaque microbiota.

Gender variability affects colonization and growth of microflora in the oral cavity. Males were the predominant gender and accounted for 71.3% (n=72) of the populace. Male predominance could be due to the observed predominance of male patients in the OPD on the data collection day, coupled with a lower participation rate among females for oral cavity sampling. However, numerous gender-related factors have been identified which may affect the microflora. There may be a hormonal influence, immune response, and behavioral factors; like oral hygiene practices and tobacco use, lifestyle factors, influence of systematic diseases, and disease susceptibility of males and females [17].

The finding that 80.2% (n=81) of participants belong to the middle class, followed by lower and upper classes, underscores the significant representation of middle-income individuals in this study. Factors such as education level and income influence oral hygiene indicators, with higher SES often correlating with improved oral health. The observation that the highest plaque index recorded was grade 2, 43.6% (n=44), indicating a moderate accumulation of plaque deposits, followed by grade 3 i.e. severe plaque accumulation in 29.7 %(30) individuals suggested that the study participants exhibited noticeable plaque buildup.

The thickness of the plaque may be related to Socioeconomic status of the studied sample. As majority of patients belongs to middle class, they have insufficient funds and resources to access regular dental visits and professional cleaning of plaque which may leads to increasing the thickness of plaque and subsequent manifestations like dental caries and periodontal problems. Lack of awareness and motivation related to oral hygiene maintenance also played a crucial among individuals.

Gram positive cocci were the predominant species followed by Gram positive bacilli. Alghamdi in KSA, also demonstrated that positive species account in greater proportion to Gram-negative species [18].

The study revealed, beta-hemolysis was the most prevalent hemolytic pattern, observed in 34.7% (n=35). Culture conditions, medium composition, and bacterial genetic variables can all affect hemolysis variability. Understanding these patterns is crucial, as certain hemolytic activities, particularly beta-hemolysis, have been associated with pathogenicity in systemic infections [19]. Therefore, recognizing the prevalence of these hemolytic types in oral isolates may provide insights into their potential roles in oral and systemic diseases.

The evaluation of the biochemical components is fundamental to understanding microbial behavior, pathogenic potential, and ecological interactions within the oral cavity. It can serve as markers for identifying high-risk organisms, predicting disease progression, or developing targeted preventive and therapeutic strategies.

This study encompasses diversified bacterial phyla i.e. Firmicutes (65.7%), Actinobacteria (22.7%), and Proteobacteria (6%). It could be due to age, gender, SES, occupation, habits, oral hygiene practices, geographical location, and many other factors. This is consistent with the study conducted in South Florida demonstrated that Firmicutes followed by Bacteroidota, Actinobacteriota, and Proteobacteria were the predominant species from oral isolates [20].

The distribution of oral bacterial species in this study was found to be statistically significantly correlated with a number of demographic and biochemical parameters. The statistically significant factors include Gender Age Occupation, Hemolysis on blood agar, Bile esculin hydrolysis Catalase, Oxidase, ONPG, CIT, MALO, ODC, VP, GEL, Nitrate, Sucrose, Mannitol, Melibiose and Raffinose. For instance, age and gender affect the overall composition of the oral microbiome, most likely as a result of hormonal shifts and lifestyle-related determinants [21]. Occupation may also affect oral bacterial diversity and microbial exposure, particularly in people who often interact with the public or work in healthcare [22]. The hemolysis pattern of bacteria was significant among species (p=0.000). In order to distinguish between oral *Streptococci* and *Staphylococci*, hemolysis patterns on blood agar were essential. Beta-hemolytic species, like *Streptococcus anginosus*, are linked to the development of abscesses, whereas alpha-hemolytic species, like *Streptococcus mitis*, are linked to health [23, 24]. Similarly, bile esculin and oxidase catalase tests were also significant (p value=0.040,0.030, 0.000) respectively. A biochemical test that helped differentiate *Enterococcus faecalis* and *Enterococcus faecium* from other Gram-positive cocci was bile esculin hydrolysis. *Neisseria mucosa* (oxidase-positive) and *Staphylococcus aureus* (catalase-positive) were distinguished from other genera using catalase and oxidase assays[25]. Catalase and oxidase responses were essential for classification; *Neisseria mucosa* was identified by oxidase activity, while *Staphylococcus* species were distinguished from *Streptococcus* by catalase positive. Particularly instructive were studies for the utilization of carbohydrates, including ONPG, citrate (CIT) consumption, mannitol, melibiose, raffinose, and sucrose fermentation. The acidogenic capability of lactose-positive species, such as *Citrobacter freundii* and *Lactobacillus casei,* was supported by positive ONPG and sucrose tests. *Staphylococcus aureus* and *Staphylococcus epidermidis* were separated by mannitol fermentation tests like malonate utilization (MALO), ornithine decarboxylase (ODC), and gelatin hydrolysis (GEL) which helped to characterize Gram-negative bacilli such as *Enterobacter gergoviae* and *Citrobacter freundii*. Voges-Proskauer (VP) positivity was notable in Enterobacteriaceae isolates, reflecting acetoin production pathways. Nitrate reduction was common among facultative anaerobes like *Rothia mucilaginosa* and *Actinomyces viscosus* [26].

The small sample size, exclusion of uncultivable bacteria due to the use of culture-dependent techniques lack of plaque thickness evaluation, lack of incorporation of advanced molecular techniques, lack of assessment of environmental factors that may influence biofilm formation, and crossectional design were the limitations of study.

It has been recommended awareness of public, better access to dental care, and encouragement of healthy lifestyle choices. Future research should embrace next-generation sequencing (NGS) and metagenomics approaches for a complete perspective on the biodiversity of oral microbiomes and their contribution to dental diseases. Longitudinal studies monitoring bacterial colonization dynamics over time would also enhance understanding of plaque biofilm development and pathogenesis.

CONCLUSION

Multiple and variable Gram-positive and negative bacterial species have been isolated from supragingival dental plaque of local isolates. The demographic, phenotypic, and biochemical characteristics demonstrated statistically significant associations with the identified organisms. It suggests that the understanding of oral health-related taxa must include not only common species but also less-common bacteria. The results highlight the importance of preventive approaches to improve oral health and prevent chronic infections.

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