ORIGINAL ARTICLE

CONTENTS

Identification of oral bacteria in patients with dentulous, partially edentulous and edentulous: A comparative study

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ABSTRACT

Aim: To investigate distribution of oral bacteria among dentulous, partially edentulous, and edentulous patients while examining influence of age and gender on these conditions. It was designed to identify the prevalence of specific bacterial species in the oral cavity and their association with different dental statuses.

Materials and Methods: Samples were taken by rinse the mouth of 66 subjects divided into 3 groups: Dentulous n=49, Edentulous n=43, Partially Edentulous n=58. Ten types of bacteria were analyzed using gram staining and biochemical tests.

Results: Staphylococcus aureus is most prevalent in the edentulous group, accounting for 27.91%, compared to 15.52% in the Partially Edentulous and 10.20% in the dentulous group. This suggests a possible increase in its presence with tooth loss. *Staphylococcus albicans* and *Streptococcus* are more common in the Dentulous and Partially Edentulous groups, with the dentulous group having the highest percentage of *Staphylococcus albus* at 22.45%. Diplococcus pneumoniae shows an increased frequency in the edentulous group 16.28% compared to the other groups, which may indicate a higher risk of pneumonia-related bacteria in patients without teeth. There was a significant association between age and tooth loss. Gender did not show any relationship neither to tooth loss nor to oral cavity bacteria.

Conclusions: Edentulism was most prevalent in individuals aged 50 years and older, emphasizing the role of aging in tooth loss. No significant gender differences were observed, indicating equal impact on males and females. Certain bacteria, like *Staphylococcus aureus* and *Streptococcus*, were more common in edentulous patients.

KEY WORDS: oral bacteria, dentulous, edentulous patients

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INTRODUCTION

Oral microbiome plays a crucial role in maintaining oral health and influencing various systemic conditions. In the context of Iraqi patients, the composition and diversity of the oral microbiome can vary significantly among edentulous, edentulous, and dentulous individuals. Oral pathogenic bacterial species participate in the pathogenesis of both caries and periodontal disease. Oral pathogens engage in negative interactions with the host, passing information, immune response, and even metabolic condition back and forth through systemic organs and the oral cavity. The oral cavity presents a critical point of contact with the external environment for the human body. Because of different microenvironments, microbial compositions among different sites within the oral cavity also differ and are very dynamic due to an intricate input of signals coming from hosts and external ecological factors [1]. Certain oral bacterial species have been linked to specific systemic conditions, for instance, bacterial osteomyelitis, aspiration pneumonia, and endocarditis in infants, preterm low birth weight, and cardiovascular disease in the oral cavity might be considered an ideal incubator because it possesses a temperature of approximately 35 to 36°C and has an abundance of moisture, an excellent supply of various types of foods and difference in oxygen tension [2]. The number of microorganism that can be removed from oral cavity by mouth rinsing various though the day. It has been observed that bacterial counts are highest in the morning on raising. As a result of eating breakfast, brushing the teeth and rinsing the mouth, these numbers decrease. A gradual increase is noted before the mid day meal after the meal a decrease Occurs. A pattern of an increase followed by a decrease is after [3]. The overall balance of the oral flora may be related to the fact that the main food source for the microorganisms is mainly saliva, epithelial cells and inflammatory exudates (gingival crevicular fluid) from the environment. These substances flow continuous-

ly through the oral cavity, removing food particles, bacteria and their waste products, equivalent to a continuous culture device containing a mixed microbial community. Irregular exposure to additional nutrients and inhibitors in human food may even cause bacterial growth to undergo a kind of "synchronization" [4]. The oral microbiome has been consistently found of major importance to oral health. This is best epitomized by Marsh (2010), who discusses the role of dental plaque biofilms in oral health and caries, arguing that dysbiosis would accrue to several oral diseases. This foundational knowledge needs to be further explored in understanding how different patient groups might present unique microbial profiles in edentulous, un-edentulous, and dentulous cases [5]. Hypothetically, it would be expected that edentulous patients would portray a far greater alteration in their oral microbiome as compared to their dentulous counterparts. Mertens et al., (2012) reported long-term results of implant-supported rehabilitation in edentulous patients and noted the problems of microbial colonization and peri-implantitis in this context. The complete lack of teeth led to distinctly varied microbial conditions, requiring targeted therapeutic measures [6]. In contrast to the above study, Jiang et al. (2019) studied the oral microbiome in elderly patients with dental caries as well as health and established a much unique microbiome composition associated with the dental status. The current research supports the theory that the presence of teeth critically influences microbial diversity and composition [7]. The microbiome dynamics in edentulous and dentulous patients also seem to differ. For example, Schulz et al., (2019) took a comparison of the oral microbiome on generalized aggressive periodontitis patients with subject's periodontitis free disclosing considerable variations which could help in understanding of the risk of periodontal disease among dentulous patients [8].

AIM

The study aimed to investigate the distribution of oral bacteria among dentulous, partially edentulous, and edentulous patients while examining the influence of age and gender on these conditions. It sought to identify the prevalence of specific bacterial species in the oral cavity and their association with different dental statuses. Additionally, the study aimed to assess the statistical significance of these relationships to provide insights into the role of oral hygiene, aging, and bacterial colonization in the progression of tooth loss and associated infections, ultimately contributing to improved preventive and therapeutic approaches.

MATERIALS AND METHODS

SAMPLING

A total of 150 salivary samples were collected in this study. The samples were collected from individuals who are attending the medical centers in Abo-Garib. Meanwhile, such samples were equally distributed to both sexes. To each sample, it involved a special form to be filled concerning the name, age, sex, and date of sampling. Materials and Methods: Mouth sample: The subjects were grouped into dentulous n=49, edentulous n=43 and partially edentulous n=58. A 5ml sterile distilled water was poured into the mouth of the subject who had rinsed his/her mouth vigorously and sampled mouth after rinsing in sterile tubes. Components were further individually cultured on BHI broth 15 ml at 37°C for 24h then after incubation was sub-cultured on blood agar and MacConkey agar and later step went for final identification of suspected isolates.

IDENTIFICATION OF BACTERIAL ISOLATES

MICROSCOPICAL EXAMINATION

Samples processed for microscopical examination using Gram stain, a loop full of bacterial isolates was fixed on microscopic slide, then smears were allowed to dry and all fixed smears were stained by Gram stain to examine cell shape, grouping, size and Gram reaction.

BIOCHEMICAL TESTS

CATALASE TEST

A single colony of each bacterial isolate was placed onto a clean glass microscopic slide using a sterile toothpick, a drop of 3% H_2O_2 was added, and the appearance of bubbles indicated a positive result.

OXIDASE TEST

A sterile wooden stick of suspected bacterial isolates was picked up from the slant growth and smeared on filter paper with a drop of freshly prepared oxidase reagent (tetramethyl-para-phenyl-diamine dihydrochloride). Positive: The development of purple colour within 5-10 seconds.

GROWTH ON MANNITOL SALT AGAR MSA

A suspected colonies were inoculated on sterile MSA and incubated for 24-48 hrs, after incubation, changing the color from red to yellow is an indicator of mannitol fermentation.

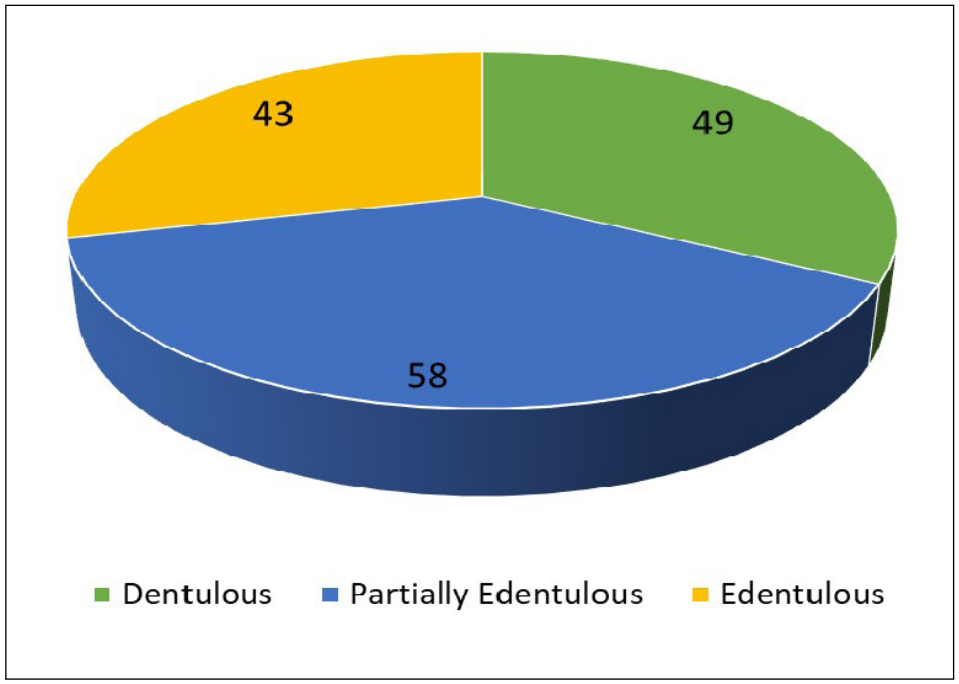

Fig. 1. Distribution of the three groups of patients.

Table 1. Growth of bacteria on blood agar

Types of bacteria	Hemolysis	Catalase	Coagulase	Mannitol
Staph. aureus	B-hemolysis	Positive	Positive	Positive
Staph. albicans	Negative	Positive	Negative	Negative
Staph. saprophyticus	Negative	Negative	Negative	Positive

Table 2. Distribution of age and sex in the three groups of patients

Types of bacteria		Dentulous (n=49)		Pat. Edentulous (n=58)		Edentulous (n=43)		Chi Square	P value
		Freq.	Percent.	Freq.	Percent.	Freq.	Percent.		
Age/ Years	20-29	15	30.61	2	3.45	1	2.33	69.77	0.000 HS
	30-39	14	28.57	3	5.17	3	6.98		
	40-49	13	26.53	23	39.66	2	4.65		
	≥ 50	7	14.29	30	51.72	37	86.05		
Gender	Male	25	51.02	31	53.45	24	55.81	0.21	0.89 NS
	Female	24	48.98	27	46.55	19	44.19		

HS: High Significant at P value <0.01; NS: Non-significant at P value >0.05.

GROWTH ON BLOOD AGAR

Bacterial isolates were inoculated on human blood agar medium prepared and incubated at 37°C for 24-48 hrs., as shown in table 1. The presence of clear zones or green around the colonies represented hemolysis of blood [4].

COAGULASE TEST

A milliliter of the human plasma was placed into two small test tubes, to the first one 0.1 ml of an overnight broth culture of suspected isolate, while nothing tube added, both tubes incubated for 4 hours or overnight. Clot formation indicated coagulase producing isolate as positive result while the second tube used as a control.

DNASE TEST

A loop of suspected isolate from a primary culture was picked up with a sterile bacteriological needle and streaked by the needle-point method on the surface of DNase agar plate. The DNase plate was then incubated at 37°C for 18-24 hours. After incubation, the plate was flooded with 1N Hcl. The appearance of the clear zone around the growth was an indication for DNase production and a positive result.

Table 3. Distribution of detected bacteria in the oral cavity of the three groups of patients

Types of bacteria	Dentulous		Pat. Edentulous		Edentulous		Chi Square	P value
	Freq.	Percent.	Freq.	Percent.	Freq.	Percent.		
Streptococcus	10	20.41	11	18.97	4	9.30	29.39	0.043 S
E. coli	5	10.20	5	8.62	5	11.63		
Klebsiella Spp.	0	0.00	5	8.62	3	6.98		
Proteus	2	4.08	1	1.72	0	0.00		
Pseudomonas	8	16.33	5	8.62	3	6.98		
Staph. aureus	5	10.20	9	15.52	12	27.91		
Staph. albus	11	22.45	8	13.79	2	4.65		
Neisseria Spp.	0	0.00	2	3.45	4	9.30		
Lactobacillus Spp.	0	0.00	5	8.62	3	6.98		
Diplococcus (Pneumonia)	8	16.33	7	12.07	7	16.28		

S: Significant at P value <0.05.

Table 4. Association between detected bacteria and gender in dentulous group

Types of bacteria	Male		Female		Chi Square	P value
	Freq.	Percent.	Freq.	Percent.		
Streptococcus	5	10.20	5	10.20	2.8	0.83 NS
E. coli	4	8.16	1	2.04		
Klebsiella Spp.	0	0.00	0	0.00		
Proteus	1	2.04	1	2.04		
Pseudomonas	4	8.16	4	8.16		
Staph. aureus	2	4.08	3	6.12		
Staph. albus	4	8.16	7	14.29		
Neisseria Spp.	0	0.00	0	0.00		
Lactobacillus Spp.	0	0.00	0	0.00		
Diplococcus (Pneumonia)	4	8.16	4	8.16		

NS: Non-significant at P value >0.05.

UREASE TEST

This was examined by streaking the surface of sterile urea agar slants with the tested bacteria and incubated at 37°C for 24 hrs. Pink color development confirmed the positive test result.

OPTOCHIN TEST

For pneumococci, they are gram positive-diplococci cultured on blood agar and then put optochin disc, only pneumococci give positive reaction (Optochin inhibit the growth of pneumococci).

RESULTS

The pie chart visually illustrates the proportional distribution of the three patient groups: dentulous, partially edentulous, and edentulous. The chart effectively shows that the groups are not equally distributed, with partially edentulous patients appearing to form the largest portion 58%, followed by the dentulous 49% and edentulous groups 43% (Fig.1).

Table 2 presents the distribution of age and sex across three groups of patients categorized as Dentulous, Partially Edentulous, and Edentulous, with a chi-square test applied to determine the significance of differences among these groups. There is a significant association between age and the type of patient group, as indicated by the chi-square value 69.77 and highly significant p-value < 0.001. The chi-square test for gender distribution reveals no significant association between gender and patient group, p = 0.89.

Table 3 illustrates the distribution of detected bacteria in the oral cavity across three groups of patients: Dentulous, Partially Edentulous, and Edentulous, with a chi-square test showing significant variation in bacterial distribution, p = 0.043. Staphylococcus aureus is most prevalent in the edentulous group, accounting for 27.91%, compared to 15.52% in the Partially Edentulous and 10.20% in the dentulous group. This suggests a

Table 5. Association between detected bacteria and sex in partially edentulous group

Types of bacteria	Male		Female		Chi Square	P value
	Freq.	Percent.	Freq.	Percent.		
Streptococcus	5	8.62	6	10.34	5.83	0.75 NS
E. coli	2	3.45	3	5.17		
Klebsiella Spp.	3	5.17	2	3.45		
Proteus	1	1.72	0	0.00		
Pseudomonas	2	3.45	3	5.17		
Staph. aureus	4	6.90	5	8.62		
Staph. albicans	5	8.62	3	5.17		
Neisseria Spp.	1	1.72	1	1.72		
Lactobacillus Spp.	2	3.45	3	5.17		
Diplococcus (Pneumonia)	6	10.34	1	1.72		

NS: Non-significant at P value >0.05.

Table 6. Association between detected bacteria and sex in edentulous group

Types of bacteria	Male		Female		Chi Square	P value
	Freq.	Percent.	Freq.	Percent.		
Streptococcus	5	10.20	6	12.24	3.14	0.92 NS
E. coli	2	4.08	3	6.12		
Klebsiella Spp.	3	6.12	2	4.08		
Proteus	1	2.04	0	0.00		
Pseudomonas	2	4.08	3	6.12		
Staph. aureus	4	8.16	5	10.20		
Staph. albicans	5	10.20	3	6.12		
Neisseria Spp.	1	2.04	1	2.04		
Lactobacillus Spp.	2	4.08	3	6.12		
Diplococcus (Pneumonia)	6	12.24	1	2.04		

NS: Non-significant at P value >0.05.

possible increase in its presence with tooth loss. *Staphylococcus albicans* and *Streptococcus* are more common in the Dentulous and Partially Edentulous groups, with the dentulous group having the highest percentage of *Staphylococcus albus* at 22.45%. *Diplococcus pneumoniae* shows an increased frequency in the edentulous group 16.28% compared to the other groups, which may indicate a higher risk of pneumonia-related bacteria in patients without teeth.

Regarding the distribution of detected bacteria according to sex subgroups of patients, the table 4 presents the distribution of various bacterial isolates among male and female patients (dentulous group), detailing both frequency and percentage. Identical frequencies and percentages for *Streptococcus* and *Pseudomonas* were observed in both genders, at 10.2% and 8.16%, respectively. In contrast, *Staphylococcus albus* showed a significant disparity, being more common in females at 14.29% compared to 8.16% in males. *E. coli* was more prevalent among males, with a frequency of 8.16%, while it was only 2.04% in females. According to the Chi-square test, there are no statistically significant differences between the distributions of males and females (P = 0.83), suggesting that gender does not have a significant impact on bacterial occurrence in this study.

The distribution of bacteria among male and female patients with partial edentulism was the focus of the current study (Table 5). Among males, *Streptococcus* and *Diplococcus* (*Pneumonia*) were found more frequently, at rates of 8.62% and 10.34%, respectively, while in females, the rates were 10.34% and 1.72%. Notably, *Staphylococcus aureus* exhibited a slightly higher occurrence in females (8.62%) compared to males (6.90%). Interestingly, both genders showed equal detection of *Klebsiella spp*., *Pseudomonas*, and *Lactobacillus spp*., whereas *Proteus* was exclusively identified in males. The chi-square analysis indicated no statistically significant association (P = 0.75) between the detection of bacteria and sex, suggesting that there is no gender-based predisposition in the bacterial distribution for this group.

These results are consistent with the overall pattern of bacterial colonization observed in partially edentulous individuals.

The present study examined the link between bacterial presence and sex within the edentulous population (Table 6). The most commonly found bacterium was *Streptococcus*, identified in 10.20% of males and 12.24% of females. In a similar vein, *Staphylococcus aureus* and *Staphylococcus albicans* exhibited marginally higher occurrences in females (10.20% and 6.12%, respectively) compared to males (8.16% and 10.20%). Interestingly, *Diplococcus* (*Pneumonia*) was observed more frequently in males (12.24%) than in females (2.04%), and *Proteus* was exclusively detected in males (2.04%). The Chi-square analysis revealed no statistically significant differences (P = 0.92) between genders, indicating that there is no variation in bacterial colonization based on sex among edentulous individuals. This points to a generally consistent distribution of bacterial species across genders in this demographic.

DISCUSSION

From the birth the oral cavity is exposed to many different microorganisms present in the local and geographic environment. Those microorganism that become oral residents are favored by the nutritional and physiologic conditions are not inhibited by the mechanical and antagonistic mechanisms of the oral cavity. The effect of different oral environments over a long period of time will result in the selection of microorganisms best suited to survive in specific areas of the oral cavity. Microbial association nearly always exists even though different types may occupy separate niches differing perhaps in only one influential environmental factor. Only the aerobic oral microbial flora were studied and isolated 10-types of bacteria according to the media and the facilities from three groups. The numerical increase of the streptococcus genus in the partially edentulous group emphasizes the fact that streptococci constitute a large and complex group of bacteria having widely varying characteristics and that under certain conditions of independent pathogenicity, they may cause a wide specific disease alone [3]. The enterobacteriacae group are usually to be only transiently present in the normal human oral cavity and their numbers are so small [9]. The increasing in some kinds depend on different factors one of them loosing the teeth some of them found sporadically and in small number without producing pathological changes [10], like Klebsiella spp. And other have very limited ability to invade the body unless the defenses are not fully developed. The pseudomonas increased in dentulous group and this in agreement with the finding of Curtin et al. [11]. The proteus spp. also increased in dentulous group and support the finding of Lederman et al. [12]. For the *genus staphylococcus* the non pathogenic spp. (*Staph* albus, and Staph saprophyyticus) increased in dentulous group in this due to the amount of carbohydrates in take [4], while the pathogenic spp. (*Staph*. *aurcus*) increased in edentulous group. Neisseria spp. Increase in the edentulous group, this type of *Neisseria* is not pathogenic, this micro organism has been found at several sites in the oral cavity including the lip, tongue, cheel, plaque and saliva, and they do not appear to have an unusual affinity for any of those oral surface [13]. The number of *Neisseria* organism on the tongue and buccal mucosa increase during the deciduous tooth eruption. *Lactobacilli* increased in the partially edentulous group. *Lactobacilli* reponedly from only a small minority if the plaque micro flora. It was found that the insertion of dental splints for 3-hours in the mouth will result in 1.49-fold increase in the *Lactobacilli* count in an oral wash Specimen Oinsi, (1962) [14]. Galofré et al. (2018) investigated the effect of *Lactobacillus* reuteri on mucositis and peri-implantitis, suggesting potential therapeutic applications for managing dysbiotic conditions in both edentulous and dentulous populations [15]. Furthermore, recent findings by Abdelbary et al. (2022) illustrate the relationship between salivary and fecal microbiome dysbiosis in inflammatory bowel disease, which may also extend to oral health conditions. This suggests that systemic health conditions can further complicate the oral microbiome landscape in dentulous and edentulous patients [16]. *Diplococcus Pneumonia* increase in the dentulous group and this confirm the study in Chicago [17], who found that about less than 20% of young normal adults 18 to 30 years of age carry pneumococci in the oral cavity. The absence of the more pathogenic pneumococci from the oral cavity may be the result of the excretion of antipneumococcal IgA antibody into the saliva [18]. Various other types of microorganisms may exist as an oral flora, but only those 10 types were available based on the equipment and supplies. The removal of the teeth will alter the environmental condition of the mouth (e.g., PH temperature, humidity, etc.) this will change or affect the number and the types of bacteria according to the new environment that will create [19].

CONCLUSIONS

The study highlights significant associations between age, bacterial prevalence, and oral health conditions across dentulous, partially edentulous, and edentulous groups. Edentulism was most prevalent in individu-

als aged 50 years and older, emphasizing the role of aging in tooth loss. No significant gender differences were observed, indicating equal impact on males and females. Certain bacteria, like *Staphylococcus aureus* and *Streptococcus*, were more common in edentulous patients, underscoring the need for targeted antimicrobial strategies. The findings stress the importance of preventive care, age-specific interventions, and comprehensive oral health programs to mitigate tooth loss and manage bacterial infections effectively.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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