Isolation and Identification of Bacteria Isolated from the Oral Cavity of Oral Leukoplakia

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Abstract: A potentially cancerous condition called oral leucoplakia (OL) is typified by white patches on the oral mucosa. Microbial colonization, especially by bacteria, has a role in the pathophysiology of OL by promoting inflammation and the advancement of the illness. In this study, bacterial species from the oral cavity of patients with OL will be isolated, identified, and compared to those found in healthy persons. From July to December 2024, samples were taken from dental clinics and specialty centers in the Diyala Governorate. Micrococcus luteus, Kocuria kristinae pneumonia, Streptococcus alactolyticus, Staphylococcus hominis, and Rothia dentocariosa were among the most commonly isolated bacterial species. The development and malignant transformation of oral leukoplakia may be facilitated by the presence of particular pathogenic microorganisms. Thirty of the 120 MDR bacterial samples showed antibacterial sensitivity, indicating a possible role in chronic inflammation and the development of disease. To better understand how these bacteria contribute to the pathophysiology of OL and create focused treatment plans, more investigation is needed.

1 INTRODUCTION

White spots that cannot be wiped off and cannot be linked to any other recognized condition are the hallmark of oral leucoplakia (OL), a frequent and possibly malignant disorder of the oral mucosa [1]. The aetiology of OL is complex and includes microbial infections, persistent irritation, alcohol and tobacco use, genetic predisposition, and artificial feeding of diabetic infants. [2]. Bacteria stand out among the microbial components because of their propensity to contribute to malignant transformation, change the oral microenvironment, and promote inflammation. The prevalence of oral leukoplakia is 4.11% worldwide [3].

The human oral cavity hosts a diverse microbiome, with both commensal and pathogenic bacteria playing significant roles in oral health and disease. Previous studies have suggested that bacterial dysbiosis in oral leukoplakia could be associated with increased inflammatory responses, oxidative stress, and immune modulation, which may facilitate the progression of precancerous lesions [4]. Oral leucoplakia has a malignant transformation rate of 7.5% based on clinicopathological investigations and 9.7% based on systematic review research. Further histological analysis revealed that some tissue portions had changed into malignant lesions, even though some leucoplakia instances exhibited clinically benign characteristics in clinical investigations. [3].

According to some research, leucoplakia is linked to an increased risk of upper gastrointestinal cancers since it is a precancerous lesion that, if ignored, can have systemic health repercussions. Identifying and isolating bacterial species from OL lesions can reveal their potential role in disease progression, aid in developing targeted therapies, and assess antibiotic resistance patterns for effective treatment planning and infection control [5]. Because oral leukoplakia has serious side effects, such as tissue abnormalities and pharmacological side effects, it must be treated as soon as possible. Traditional techniques such as local surgical excision and systemic medication application have been supplanted by cryotherapy, laser, and photodynamic therapy. Both men and women are more prone to have oral leukoplakias, but they are also more likely to develop into malignant transformations. Additionally, nonsmokers are more vulnerable. [6].

This study aims to isolate and identify bacterial species from the oral cavity of patients with oral

leukoplakia, compare them with bacteria present in healthy individuals, and assess their antibiotic susceptibility profiles. By doing so, we hope to highlight the potential microbial factors involved in OL pathogenesis and provide a foundation for future research in microbial-targeted therapies at follow.

2 MATERIALS AND METHODS

2.1 Sample Collection

The study was conducted at the Laboratory Biology Department, /College of Education for Pure Sciences/University of Diyala and the First Specialized Dental Center in the Ba'aqubah / Diyala Governorate. The time frame is September 2024–December 2024. Samples were taken from gingivitis patients. A cotton swab was used to gather them from both sexes' mouth cavities. Twenty percent of the samples exhibited no growth, whereas 120 (80%) of the samples showed positive growth. A total of 55 (46%) male and 65 (45%) female samples received the positive growth isolates. At the First Specialized Dental Centre in Baqubah/Diyala Governorate, they were between the ages of one and forty. The plates were incubated at 37 degrees Celsius for 24 hours while they were cultivated on a selective and differential medium, such as blood agar and MacConkey agar.

2.2 Isolation and Diagnosis

Direct culture of the materials was performed on a nutrient agar medium. Following purification on Deferential blood agar and MacConkey selective culture medium, the isolates were aerobically cultured for 24 hours at 37° C. Phenotypic and biochemical assays were then performed, and the Vitek 2 compact was used for confirmation

2.3 Microscopic and Biochemical Examination

Following the conclusion of the Gram staining procedure, a microscopy examination is conducted. A 100X objective is used to examine the slide. Following the identification of the gram stain-positive and gram-negative bacterial kinds, all biochemical assays were carried out and the bacteria were cultivated on diagnostic and differential media tailored to each type of bacteria [6].

3 RESULTS

150 gingivitis patients had their samples taken. Specialized dentistry facilities provided the samples. As seen in Table 1 and Figure 1, there was a significant difference ($p<0.05$) between the number of samples that showed positive growth (120, or 80%) and those that did not (30, or 20%).

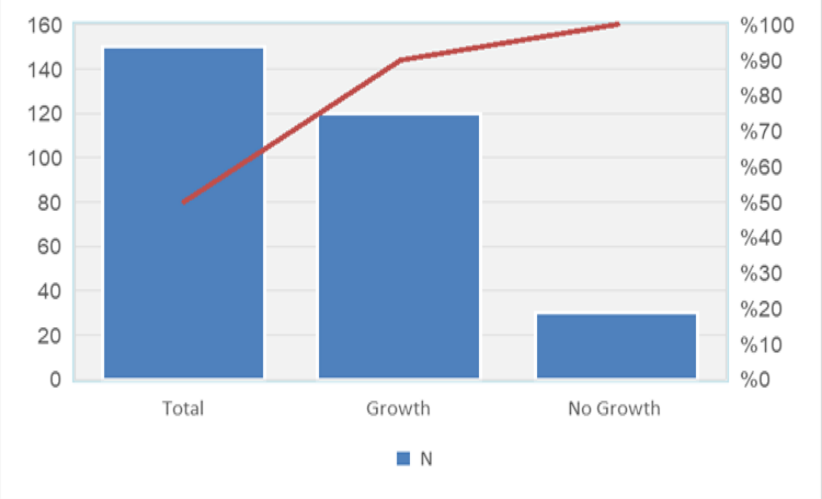

Figure 1: Participants' bacterial growth frequency and percentages.

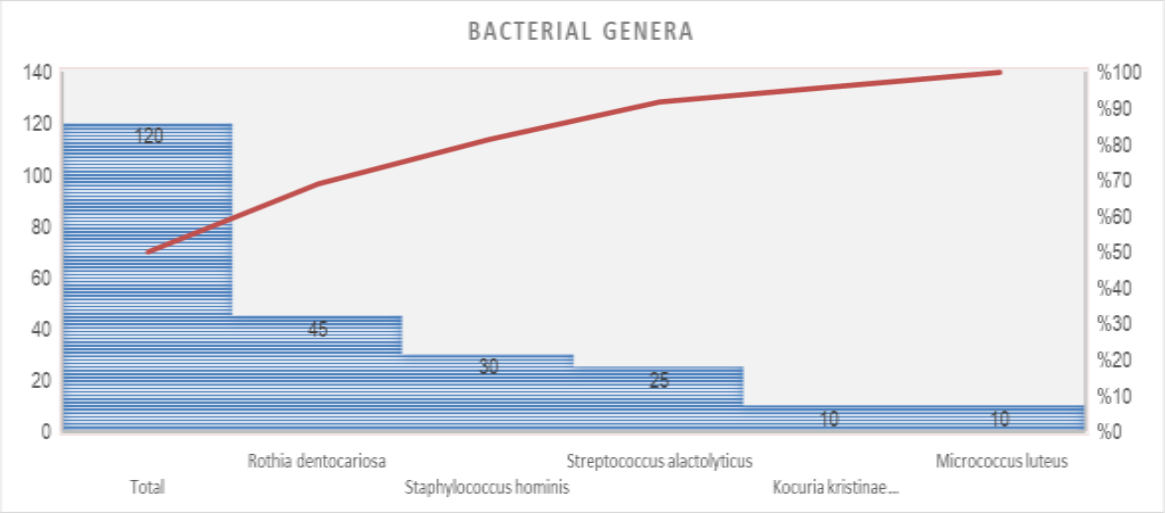

Figure 2: Participants' bacterial species frequencies and percentages.

Table 1: Frequency and percentages of bacterial growth in participants.

Bacteria growth	No.	%	P Vule
Growth	120	80%	P<0.001***
No Growth	30	20%	
Total	150	100%	

A range of age groups for both sexes were included in the sampling. The following is the distribution of the isolates that showed favorable growth: Table 2 displays 55 (46%) male samples and 65 (54%) female samples.

Table 2: Distribution of participants with bacteria growth according to gender.

Gander	N	%	P value
Males	55	46%	p>0.05
Females	65	54%	
Total	120	100%	

Present findings showed the most bacterial growth was in patients within age groups; 1-10 (32.5%) and >40 years (36.7 %) and a little of it was at 11-20 (10.0 %), 21-30 years (12.5 %), 31-40 years (8%). The differences in bacterial growth among age groups were significant(p<0.05) Table 3.

The following were the findings of the investigation that demonstrated the presence of bacterial growth in the samples: Micrococcus luteus 10 (8%), Kocuria Kristine pneumoniae 10 (8%), Streptococcus alactolyticus 25 (21%), Staphylococcus hominis 30 (25%), and Rothia dentocariosa 45 (38%). There were substantial differences across the types of bacteria (p<0.05). Figure 2 and Table 4.

Table 3: Distribution of participants with bacterial growth according to age groups.

Age group	No.	%	P Value
1-10	39	32.5%	*p<0.001***
11-20	12	10.0%	
21-30	15	12.5 %	
31-40	10	8.3%	
40<	44	36.7 %	
Total	120	100%	

Table 4: Frequency and percentages of bacterial species in participants.

Bacterial genera	No.	%
Rothia dentocariosa	45	38%
Staphylococcus hominis	30	25%
Streptococcus alactolyticus	25	21%
Kocuria kristinae pneumoniae	10	8%
Micrococcus luteus	10	8%
Total	120	100%
P Value	***P<0.001	

4 DISCUSSIONS

The oral cavity of the human is a mini ecosystem comprised of different niches colonized by an immense number of microorganisms, including fungi, several types of viruses and diverse bacterial

fauna. Around 1100 different taxa were discovered in the oral cavity and recorded in the Human Oral Microbiome Database [7]. The most prevalent potentially cancerous condition affecting the oral mucosa is oral leukoplakia. The role of bacterial colonization in the progression of leukoplakia and its possible development into oral squamous cell cancer. The isolation and identification of bacteria from oral leukoplakia lesions, as well as their possible role in the development of the disease, were the main objectives of this investigation [8].

This study investigating the microbial composition of oral leukoplakia lesions has identified several bacterial genera with increased prevalence. Notably, Rothia dentocariosa 45 (38%), Staphylococcus hominis 30 (25%), Streptococcus alactolyticus 25 (21%), Kocuria Kristine pneumonia 10(8%), and Micrococcus luteus 10(8%). The differences among bacterial species were significant ($p<0.05$) and were found in higher abundance in these lesions compared to healthy oral tissues. The highest percentage of bacteria isolated from loral leukoplakia cases was Rothia dentocariosa 45(38%). Tables 3-4. These findings suggest a potential association between these bacteria and the pathogenesis of oral leukoplakia. Gram-positive, spherical to rod-shaped bacteria, Rothia species are typically found in the respiratory and oral tracts. [9].

This study showed that the incidence rate is higher among 55(46%) males than 65 (54%) females. This study disagrees with the findings of many authors' studies, whose studies have shown that the incidence rate among males is higher than that of females, at a ratio of 2:1. These results are consistent with those of many researchers regarding the occurrence of bacterial isolates, but at varying rates. This depends on the patient's health status, as most of the isolated bacteria are opportunistic bacteria, which are found as normal flora in the mouth and then become pathogenic depending on the patient's health and immune status [10]. This study offers highlights of Rothia species, focusing on their identification, pathogenicity, clinical implications and the role of Rothia dentocariosa as both a commensal organism in the oral cavity and a potential opportunistic pathogen [11].

According to in silico research of R. dentocariosa entire genome and proteome, a number of proteins are thought to have both virulence and secretion potential. Only mild biofilms could be formed by R. dentocariosa. [12]. Depending on the stimulant being utilized, *R. dentocariosa's* capacity to produce distinct cytokines changed. Certain cytokines that were not produced by whole cells or biofilm supernatants were produced by biofilms and planktonic cultures.

Planktonic and biofilm cells produced IL-8 at almost identical levels, but only the planktonic cultures produced IL-10 at significantly greater levels ($P<0.05$). Compared to biofilm and planktonic cultures, the biofilm supernatant and whole cell stimulants produced lower quantities of cytokines. [13]. Antimicrobial susceptibility testing revealed varying resistance patterns among bacterial isolates, with some strains demonstrating resistance to commonly used antibiotics such as amoxicillin and tetracycline[14]. The presence of multidrug-resistant bacteria suggests that conventional antibiotic therapy may be less effective in managing secondary bacterial infections in oral leukoplakia patients. Given the increasing evidence linking microbial dysbiosis to oral potentially malignant disorders, future research should focus on characterizing bacterial virulence factors, host-microbiome interactions, and targeted antimicrobial or probiotic interventions to modulate the microbial environment in leukoplakia patients [15].

4 CONCLUSIONS

A wide variety of bacterial species that colonize the oral cavities of patients with oral leukoplakia were effectively identified in this investigation. Staphylococcus hominis, Micrococcus luteus, Rothia dentocariosa, Streptococcus alactolyticus, and Kocuria kristinae were the most commonly isolated organisms. Even though these bacteria are a typical element of the oral flora, they may be involved in the pathophysiology and persistence of oral leukoplakia lesions or chronic inflammation, particularly in cases of immune dysregulation or mucosal impairment. The polymicrobial character of the oral cavity and the significance of comprehensive microbial profiling in patients with premalignant oral lesions are highlighted by the discovery of both Gram-positive and Gram-negative organisms. Further molecular and antimicrobial susceptibility studies are recommended to better understand the virulence potential and resistance profiles of these isolates, particularly in the context of chronic oral mucosal conditions.

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