
ORIGINAL ARTICLE**Aerobic bacteriology of the subgingival plaque in patients with and without periodontitis undergoing tooth extraction**

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Abstract:

Aim: To report the pattern of distribution of the aerobic subgingival microflora in patients with and without periodontitis undergoing tooth extraction and healthy controls.

Methods: The prevalence and the distribution of the aerobic cultivable microflora were examined in healthy controls, patients undergoing tooth extractions without periodontitis and patients undergoing tooth extraction with periodontitis. The bacterial isolates were grown using standard culture techniques and the isolates were identified using automated Vitek2 (bioMerieux).

Results: A total of 370 aerobic isolates, comprised of 32 different species were identified in this study. Majority of the strains 225 (60.81%) were isolated from patients of periodontitis as compared to 145 (39.19%) patients without periodontitis.

Conclusions: There is a distinctive bacterial flora in the healthy oral cavity which is different from that of the patients with

periodontitis. However, there is predominance of viridans group streptococci in the subgingival plaque of healthy controls and patients as well, suggesting their contribution in progression of periodontitis, if oral hygiene is compromised.

Keywords: Periodontitis, tooth extraction

Introduction:

The oral cavity is colonized with different types of microorganisms.⁽¹⁾ Oral cavity is the initial part of the gastrointestinal tract; due to the regular supply of food it makes an environment favourable for the growth of microorganisms. The microbiology of the oral subgingival flora has been the area of intense investigation for several decades. This focus is justifiable since bacteria are the etiological agents of periodontitis and other oral diseases, which remain the primary cause of tooth loss in adults worldwide.

The largest biomass of oral bacteria in the mouth exists on the teeth, which comprises dental plaque, can accumulate upto 10¹¹

organisms per gram wet weight and bacteria form the predominant microflora of the dental plaque.⁽²⁾ Species of bacteria which are normally associated with subgingival plaque are also associated with periodontopathic pathogens such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Prevotella intermedia* in periodontal disease.⁽³⁾ It is therefore essential to gain a complete understanding of the bacteria colonising the subgingival plaque, which are associated with the periodontopathic pathogens in periodontal disease process and systemic infections such as infective endocarditis. Considering this dual relationship of the oral microflora in health and disease, it is vital to understand its composition and define its role in the oral cavity. In the present study an attempt has been made to find out the bacterial isolates commonly associated with the subgingival plaque in patients with and without periodontitis undergoing tooth extraction.

Materials and methods:

This study was approved by the institutional ethical committee. This study included 120 subjects, divided into three groups:

(Group I) 40 healthy controls

(Group II) 46 patients undergoing single tooth extraction without periodontitis.

(Group III) 34 patients undergoing single tooth extraction with periodontitis.

A written informed consent was obtained from all participants.

Exclusion criteria:

We excluded patients if they had fewer than 10 teeth; an active viral infection, poorly controlled systemic disease, penicillin allergy, antimicrobial usage within three months prior dental treatment, temperature greater than 100.5°F or facial cellulitis; or were immunocompromised by virtue of disease or medications.

Inclusion criteria:

Patients :The study was initiated with patients who were enrolled with our hospital-based dental services who needed to have at least one erupted tooth extracted.

Controls -Controls were healthy subjects who were matched for age and sex and satisfied the same exclusion criteria as the cases.

Bacteriological analysis:

A prospective bacteriological analysis of the subgingival plaque was carried out in patients and controls after taking pre-informed and written consents from the volunteers.

Clinical samples of subgingival plaque were obtained from healthy controls and patients undergoing tooth extraction. Subgingival plaque samples were collected from the gingival area of buccal and lingual tooth surfaces using sterile Gracey curettes into sterile ringer's solution.⁽⁷⁾

Subgingival plaque specimens were inoculated onto special media, Tryptone soya blood agar supplemented with strepto supplement (Nalidixic acid 3.750 mg, Nemomycin sulphate 1.060 mg and Polymixin B sulphate 8500 units for 500 ml media) and Mutans Sanguis agar (Himedia laboratories, Mumbai). Cultures with growth were further subjected to standard biochemical identification using automated Vitek 2 (bioMérieux) system to complete the strain identification.

Results:

The average age of the 120 adult subjects was 49 years, 8 months \pm 3 years, 8 months.

Subgingival plaque bacteriology:

A total of 69 bacterial strains were isolated from subgingival plaque of group I healthy controls comprising of 17 bacterial species (**Table 1**). The predominant isolates were viridans group streptococci 59 (85.51%), whereas the other bacterial strains included *Kocuria rosea* 3 (4.35%), *Gemella morbillorum* 2 (2.90%), *Kocuria cristinae* 2

(2.90%), coagulase negative staphylococci 1 (1.45) and other bacterial species.

The distribution of aerobic organisms isolated from subgingival plaque samples of patients undergoing tooth extraction without periodontitis (group II) and patients undergoing tooth extraction with periodontitis (group III) is shown in **Table 2**. Out of the 80 patients of tooth extraction, all the subgingival plaque samples (yielded positive cultures; producing 370 different isolates of which majority were 260 (70.27%) were viridans streptococci. Isolation of viridans group streptococci was highest among the Group III subjects with periodontitis 167 (45.30%), when compared to group II subjects without periodontitis 93 (25.13%). However, the rate of isolation of other bacteria was almost similar in both groups; Group III 58 (15.67%) and Group II 52 (14.05%). Overall rate of bacterial isolation was higher in Group III subjects with periodontitis 225 (60.81%) than Group II subjects without periodontitis 145 (39.18%).

Table No. 1: Distribution of bacterial strains isolated from subgingival plaque of group A-healthy controls (n=40) on aerobic culture

Sr. No	Non-streptococcal species	No.	(%)
1	<i>Kocuria rosea</i>	3	(4.35)
2	<i>Gemella morbillorum</i>	2	(2.90)
3	<i>Kocuria cristinae</i>	2	(2.90)
4	CONS	1	(1.45)
5	<i>Micrococcus</i> spp.	1	(1.45)
6	<i>Neisseriae</i> spp.	1	(1.45)
Sub- total I		10	(14.49)
Viridans group streptococcal species			
7	<i>Streptococcus oralis</i>	12	(17.39)
8	<i>Streptococcus mutans</i>	12	(17.39)
9	<i>Streptococcus mitis</i>	9	(13.04)
10	<i>Granulicatella elegans</i>	6	(8.70)
11	<i>Granulicatella adiacens</i>	4	(5.80)
12	<i>Streptococcus anginosus</i>	4	(5.80)
13	<i>Streptococcus gordonii</i>	3	(4.35)
14	<i>Streptococcus sanguinis</i>	3	(4.35)
15	<i>Streptococcus constellatus</i>	2	(2.90)
16	<i>Streptococcus parasanguinis</i>	2	(2.90)
17	<i>Streptococcus sinensis</i>	2	(2.90)
Sub- total II		59	(85.51)
Total I + II		69	(100)

Table No. 2: Distribution of microorganisms isolated from subgingival plaque of patients undergoing tooth extraction with and without periodontitis (n=80)

Microorganism isolated	Patients without periodontitis Group I (n=46)	Patients with periodontitis Group II (n=34)	Total isolates
Bacterial isolates	No. of isolates		
<i>Kocuria rosea</i>	8	11	19
<i>Enterococcus</i> spp.	5	6	11
<i>Gemella morbillorum</i>	4	7	11
<i>CONS</i>	3	8	11
<i>Corynebacterium</i> spp.	4	4	8
<i>Neisseriae</i> spp.	5	3	8
<i>Staphylococcus aureus</i>	4	3	7
<i>Kocuria cristinae</i>	3	3	6
<i>Micrococcus</i> spp.	2	4	6
<i>Bacillus</i> spp.	4	1	5
<i>Rothia</i> spp.	4	1	5
<i>Eikenella</i> spp.	1	3	4
<i>Acinetobacter lwoffii</i>	2	1	3
<i>Escherischia coli</i>	1	1	2
<i>Lactococcus</i> spp.	2	0	2
<i>Erysipelothrix rhusiopathiae</i>	0	1	1
<i>Pseudomonas aeruginosa</i>	0	1	1
sub-total I	52	58	110
Viridans Group Streptococci			
<i>Streptococcus mitis</i>	12	38	50
<i>Streptococcus oralis</i>	12	33	45
<i>Streptococcus mutans</i>	14	16	30
<i>Streptococcus anginosus</i>	2	4	6

<i>streptococcus constellatus</i>	2	5	7
<i>Streptococcus sanguinis</i>	18	23	41
<i>Streptococcus parasanguinis</i>	5	7	12
<i>Streptococcus gordonii</i>	1	3	4
<i>Streptococcus hyointestinalis</i>	0	1	1
<i>Streptococcus pluranimalium</i>	0	1	1
<i>Streptococcus sinensis</i>	1	1	2
<i>Streptococcus thoralensis</i>	0	1	1
<i>Streptococcus tigurinus</i>	0	1	1
<i>Nutritionally variant streptococci</i>			
<i>Granulicatella adiacens</i>	9	12	21
<i>Granulicatella elegans</i>	17	21	38
sub-total 2	93	167	260
Total isolates (1+2)	145	225	370

Discussion:

It has long been known that oral bacteria preferentially colonize different surfaces in the oral cavity as a result of specific adhesins on the bacterial surface binding to complementary specific receptors on a given oral surface.^(8,9) The purpose of this study was to define the predominant bacterial flora of the healthy oral cavity by identifying and comparing the cultivable bacterial species from the subgingival plaques from patients with and without periodontitis and healthy controls.

It was observed that, a total of 69 aerobic isolates from healthy controls and 370 aerobic isolates from patients undergoing

tooth extraction were recovered. Among these isolates, majority of the strains belonged to viridans group of streptococci 59 (85.51%) in controls and 260 (70.27%) in patients undergoing tooth extraction, whereas the other bacterial strains accounted for 10 (14.49%) in controls and 110 (29.73%) in patients undergoing tooth extraction (Table 1, 2).

The oral cavity can be colonized by a wide range of bacteria; more than 700 species have been detected.⁽¹⁰⁾ Viridans streptococci constitute a significant proportion of the flora around the teeth, especially in the dental biofilm that grows above the gingival crest. The supragingival plaque also contains a higher proportion of viridans streptococci

species, whereas deeper periodontal pockets harbour more anaerobic and Gram-negative species. These factors may explain our finding of high incidence of viridians streptococcal isolation from the subgingival plaque in the study population. Our findings are in agreement with many earlier studies, which have also reported predominance of viridians streptococci in the oral flora, as compared to other bacterial genera.⁽¹¹⁻¹⁶⁾

Our study also shows a significant relationship between distribution of subgingival plaque microflora and poor oral hygiene and periodontal disease parameters. High rate of bacterial isolation 225 (60.81%) was observed in patients with periodontitis, whereas the rate of bacterial isolation 145 (39.19%) in patients without periodontitis was comparatively less, these findings fairly correlate to the fact that poor oral hygiene results in plaque and calculus accumulation around teeth that can lead to inflammation and ulceration of the gingival tissues (that is, gingivitis), which precedes periodontitis and eventual tooth loss.⁽¹⁷⁾

Conclusion:

There is a distinctive bacterial flora in the healthy oral cavity which is different from that of the patients with periodontitis. However, there is predominance of viridans group streptococci in the subgingival plaque of healthy controls and patients as well,

suggesting their contribution in progression of periodontitis, if oral hygiene is compromised. Further studies are necessary to analyze larger numbers of clinical samples for the levels of essentially all oral bacteria in well controlled clinical studies; to draw concrete evidences about the distribution of bacteria in the subgingival bacterial community and their role in periodontal diseases.

Conflict of interest: None to declare

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