Detection of *Enterococcus faecalis* in subgingival biofilms of healthy, gingivitis, and chronic periodontitis subjects

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**Abstract:**

**Background:** *Enterococcus faecalis* is a Gram-positive, facultative anaerobic coccus that can survive under harsh conditions. Studies have shown a positive relationship between biofilm formation and gelE gene expression in *E. faecalis*. The production of gelatinase (MMP 2) has been detected in 50% of *E. faecalis* isolates from endodontic and periodontal infections, which suggests its role in the pathogenesis of apical and marginal periodontitis. Although *E. faecalis* is not considered a periodontopathogen, this species has been more frequently detected in subgingival samples with periodontitis than from periodontally healthy subjects, suggesting that the local conditions in periodontitis may favor its colonization. Hence, the aim of the current study was to detect and compare the presence of *E. faecalis* in subgingival biofilms of healthy, gingivitis, and periodontitis subjects. **Materials and Methods:** A total of 100 subjects aged between 25 and 55 years, from the Outpatient Department of Periodontics were recruited for the study. All the subjects were screened for gingival and periodontal status using plaque index, gingival index, and clinical attachment loss. They were divided into three groups based on the clinical findings.

- Group A: 18 healthy individuals (gingival index with score zero)
- Group B: 34 gingivitis patients (gingival index with score >1)
- Group C: 48 chronic periodontitis patients (clinical attachment loss >5 mm in >30% of sites).

Subgingival plaque samples of all the enrolled subjects were collected using a sterile curette, later poured into a transport medium (Viability Medium Goteborg Agar III) and sent for microbial culturing within 2 h for detection of *E. faecalis*.

**Results:** *E. faecalis* was detected in 26.8% of all samples evaluated. There was a significantly higher frequency of *E. faecalis* in subgingival biofilms of periodontitis group (41.7%), compared to gingivitis (5.9%) and healthy group (0%).

**Conclusion:** Enterococci may contribute to increased collagen and periodontal destruction and may further lead to disease progression in patients with chronic periodontitis.

**Key words:** Chronic periodontitis, *Enterococcus faecalis*, gelatinase, subgingival biofilm

**INTRODUCTION**

Enterococci are Gram-positive, facultative anaerobic cocci, which are resilient by nature and able to survive a wide array of hostile conditions.**1** They can persist in the environment for long periods and are known as commensals in the gastrointestinal tract. Enterococci have gained significance as they cause nosocomial infections and for their ability to resist the currently available antibiotics via horizontal gene transfer.

*Enterococcus faecalis*, a Gram-positive anaerobic coccus, has been isolated from patients with posttreatment apical periodontitis, refractory marginal periodontitis, and peri-implantitis. The ability of *E. faecalis* to cause infections has been linked to variable traits that enhance its virulence.**2**

Enterococci surface protein (ESP) was detected in most strains isolated from endodontic, periodontal, and oral infections. Since ESP has been associated with higher biofilm production of the strains, the high prevalence of ESP within oral isolates suggests that this surface protein may be a potential virulence trait that participates in the colonization of different niches of the oral cavity.**2**

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Gel E (secretory metalloprotease gelatinase E), an extracellular metalloprotease, is a genetic factor of E. faecalis with ability to hydrolyze and degrade collagen, fibrinogen, and hemoglobin. This suggests a role for this factor in the pathogenesis of apical and marginal periodontitis.

In light of this knowledge, the current study was designed to assess and compare the presence of E. faecalis in subgingival biofilms of healthy periodontium, gingivitis, and chronic periodontitis subjects.

**MATERIALS AND METHODS**

A total of 100 individuals including males and females who aged between 25 and 55 years with a minimum of 10 natural teeth and were systemically healthy with no history of antibiotics intake in the past 6 months were enrolled from the Outpatient Department of Periodontics during December 2017 to February 2018. Ethical committee clearance was obtained from the institution (SDC/SMG/2017/661-A). Consent was obtained from all the participants. Smokers, immunocompromised, pregnant, and lactating mothers, and patients with extensive dental caries and history of previous endodontic or periodontal treatments were excluded from the study.

Clinical parameters such as plaque index, gingival index, and clinical attachment loss using Williams graduated probe were assessed. Based on convenience sampling, patients with no bleeding on probing and a score zero of gingival index and no clinical attachment loss were categorized as healthy group (Group A). Patients with score one or above of gingival index were grouped as gingivitis (Group B) and those with generalized bleeding on probing and clinical attachment loss equal to or more than 5 mm, in more than 30% of sites were grouped as periodontitis (Group C). Total number of patients allotted for the study = 100 (n).

- Group A: 18 healthy individuals
- Group B: 34 gingivitis patients
- Group C: 48 chronic periodontitis patients.

**Assessment of bacteria**

Subgingival plaque samples were collected from the enrolled patients using sterile Gracey curette and were pooled in fresh transport media “Viability Medium Goteborg Agar III” which was anaerobically prepared and sterilized. Samples were then sent to laboratory for microbial analysis, culturing and identification of E. faecalis within 2 h.

**Microbial culturing**

Samples were thoroughly shaken in a mixer for 60 s, and from each sample, 1 ml was collected for culturing. From serial dilutions, 0.1 ml was inoculated and plated on anaerobic blood agar plates. It was then incubated in an anaerobic chamber at 37°C for 2 days and the colonies of E. faecalis were identified. After completion of incubation, automation was done with Vitek® II system (manufactured by Biomerieux, USA). The purity of the cultures was confirmed by further biochemical tests on the basis of Gram staining, colony morphology, esculin hydrolysis, nitrate reduction, indole production, and oxidase and catalase activities.

**Statistical analysis**

Statistical evaluation was done using SPSS system version 16-manufactured by SPSS Inc., Chicago, USA. The Kolmogorov–Smirnov and the Shapiro–Wilk test and the test of normality indicated that the data were not normally distributed. Hence, Kruskal–Wallis one-way ANOVA was used to compare the plaque index, gingival index, and clinical attachment loss in three groups and also the presence of E. faecalis between three groups.

**RESULTS**

The study reported the results of 100 individuals. Table 1a and b reveals and compares the mean plaque index score, gingival index score, and clinical attachment loss for the three groups.

Kruskal–Wallis H-test showed that overall, there was a statistically significant difference in plaque index between all the three different experimental groups, χ² (2) = 51.37, P < 0.001 with a mean rank plaque index for Group C being 68.56, for Group B being 45.21, and for Group A being 12.33. There was a statistically significant difference in Gingival Index between all three experimental groups, χ² (2) = 47.76, P < 0.001 with a mean rank gingival index for Group C being 64.31, for Group B being 52.71, and for Group A being 9.50. Kruskal–Wallis H-test also showed that there was again a statistically significant difference in clinical attachment loss between all the three experimental groups, χ² (2) = 86.32, P < 0.001 with a mean rank clinical attachment loss for Group C being 76.50 and for Group B and Group A being 26.50.

The prevalence of E. faecalis is demonstrated in Table 2. Among the samples from 18 healthy periodontium, none of them showed the presence of E. faecalis, and of 34 gingivitis and 48 chronic periodontitis patients, one and eight samples were

Table 1a: Comparison of the plaque index score, gingival index score and clinical attachment level for the three groups

| Group        | Mean | SD   | Minimum | Maximum | Quartiles | Test statistic |
|--------------|------|------|---------|---------|-----------|---------------|
|              |      |      |         |         | Q1        | Median        | Q3        | IQR  | P     |
| PI Periodontitis | 0.80 | 0.57 | 0.00    | 2.00    | 0.31      | 0.60          | 1.30      | 0.99 | χ² (2)=51.37 <0.001 |
| Gingivitis   | 0.21 | 0.25 | 0.00    | 1.00    | 0.12      | 0.26          | 0.40      | 0.28 |       |
| Healthy      | 0.017| 0.038| 0.00    | 0.10    | 0.00      | 0.00          | 0.00      | 0.00 |       |
| GI Periodontitis | 2.18 | 2.44 | 1.00    | 17.00   | 1.60      | 1.70          | 1.98      | 0.38 | χ² (2)=47.76 <0.001 |
| Gingivitis   | 1.56 | 0.33 | 0.87    | 2.10    | 1.35      | 1.60          | 1.80      | 0.45 |       |
| Healthy      | 0.00 | 0.00 | 0.00    | 0.00    | 0.00      | 0.00          | 0.00      | 0.00 |       |
| CAL Periodontitis | 1.67 | 0.43 | 0.50    | 2.37    | 1.30      | 1.70          | 2.08      | 0.78 | χ² (2)=86.32 <0.001 |
| Gingivitis   | 0.00 | 0.00 | 0.00    | 0.00    | 0.00      | 0.00          | 0.00      | 0.00 |       |
| Healthy      | 0.00 | 0.00 | 0.00    | 0.00    | 0.00      | 0.00          | 0.00      | 0.00 |       |

P value of 0.05 were considered significant. IQR – Interquartile range; SD – Standard deviation; PI – Plaque index; GI – Gingival index; CAL – Clinical attachment loss; P – Probability value; χ² – Chi squared test statistics
**DISCUSSION**

*Enterococcus faecalis* is not a normal commensal in the oral cavity; it can be conceived directly or indirectly based on the oral status. *E. faecalis* can be commonly retrieved from dorsum of the tongue (55%), gingival sulcus (22%), and oral rinse samples (29%). It can get incorporated into oral cavity by food contaminants and also by nosocomial infections. Souto and Colombo in their study on the incidence of *E. faecalis* in foods – A conundrum for food safety. Int J Food Microbiol 2003;88:105-22.

**REFERENCES**

1. Van Tyne D, Gilmore MS. Friend turned foe: Evolution of enterococcal virulence and antibiotic resistance. Annu Rev Microbiol 2014;68:337-56.
2. Pinheiro ET, Mayer MP. *Enterococcus faecalis* in oral infections. J Interdiscipl Med Dent Sci 2014;3:160.
3. Franz CM, Stiles ME, Schleifer KH, Holzapfel WH. Enterococci in foods – A conundrum for food safety. Int J Food Microbiol 2003;88:105-22.
4. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121-35.
5. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963;21:333-51.
6. Souto R, Colombo AP. Prevalence of *Enterococcus faecalis* in subgingival biofilm and saliva of subjects with chronic periodontal infection. Arch Oral Biol 2008;53:155-60.
7. Manson JM, Hancock LE, Gilmore MS. Mechanism of chromosomal transfer of *Enterococcus faecalis* pathogenicity island, capsule, antimicrobial resistance, and other traits. Proc Natl Acad Sci U S A 2010;107:12269-74.