

Original Article

Enterococci in the oral cavity of periodontitis patients from different urban socioeconomic groups

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ABSTRACT

Background: Enterococci are the transient constituents of the oral microbiome and have been now increasingly related to oral and systemic diseases. They have now become the most notable nosocomial pathogens and also been linked to etiology of periodontitis. This study evaluates the prevalence of Enterococci in the chronic periodontitis and healthy Indian cohort in different urban socioeconomic groups.

Materials and Methods: In this retrospective study, individuals in the age range of 18–75 years were included. Seventy individuals had mild-to-moderate periodontitis and 30 healthy persons were taken as controls. A questionnaire was administered. Paper point samples of gingival crevicular fluid were obtained, pooled, and sent to microbiology laboratory in a transport media. Forty-six isolates were identified as enterococci for statistical analyzes Pearson's Chi-square test used and $P < 0.001$ was considered to be significant.

Results: The prevalence of enterococci was seen mostly in the individuals from lower socioeconomic class, having poor oral hygiene, and smokers. This was significantly different from those of upper class ($P < 0.001$). The predominant species isolated was *Enterococcus faecalis*.

Conclusion: Enterococci particularly *E. faecalis* followed by *Enterococcus faecium* could play a crucial role in the severity or progression of periodontitis particularly in a favorable oral environment.

Key Words: Nosocomial, oral hygiene, periodontitis, prevalence, socioeconomic status

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INTRODUCTION

The oral health-care situation is grim in India with statistics presenting that 95% of the Indian population suffers from periodontal disease.^[1] Periodontal diseases are one of the major causes of tooth loss in India.^[2] Recently, enterococci have been implicated a lot in the pathogenesis of periodontal diseases.^[3] Enterococci are now also ranked the third-most nosocomial pathogen after coagulase-negative staphylococci and *Staphylococcus aureus*.^[4] The ability of enterococci to survive under a wide range of physicochemical

conditions, such as drying, high osmolarity, and the presence of disinfectants allows them to persist on various surfaces in hospitals and on the hands of health-care workers.^[5] The increasing emergence of antimicrobial-resistant phenotypes, particularly the vancomycin-resistant enterococci is now becoming a matter of concern.^[6,7]

The data about the oral prevalence of enterococci in the Indian population are very scant. Recently, a study has reported the population structure of enterococci human

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isolates from six different European countries.^[8] The currently available data pertain to the oral enterococci in patients having endodontic therapy. enterococci particularly *Enterococcus faecalis* has been detected in the range of 3.7%–35% in the oral microbiota of periodontitis patients suggesting that periodontal infection may favor the colonization of this species.^[9] A recent study has reported that 60% of patients with diabetes had oral *E. faecalis* and *Enterococcus faecium* as compared to 6.6% in the controls.^[10] The oral cavity could be an important reservoir of virulent, biofilm-forming, and antibiotic-resistant enterococci strains. Although enterococci are sensitive to antibiotics such as vancomycin, erythromycin, ciprofloxacin, teicoplanin, amoxicillin and gentamicin, emergence of multidrug-resistant strains is becoming a matter of concern. Recently, it has been seen that in the treatment of apical periodontitis that when in previously infected canals elimination or reduction of bacterial pathogens is done an improved prognosis is achieved.^[11,12] *E. faecalis* has been implicated as the most consistently reported organism in dental infections. The organism can survive up to a pH = 11.5, can grow as a monoinfection and remain viable in prolonged starvation.^[13,14] The reduction in bacterial counts by mechanical instrumentation such as cleaning, disinfection, oral medicaments before the procedure will improve the outcome of the procedure. Henceforth, the knowledge of the prevalence of enterococci in chronic periodontitis patients can be of great value in the treatment strategy. Therefore, the present study was planned to investigate the possible association between the prevalence of enterococci species in different urban socioeconomic groups in periodontal disease and healthy consort of Indian population and also evaluate the different clinical parameters.

MATERIALS AND METHODS

Subject population

In this retrospective study, 70 individuals in the age range of 18–75 years having chronic periodontitis visiting the Department of Periodontics, Dr Harvansh Singh Judge Institute of Dental Sciences and Hospital, Panjab University, Chandigarh, India, were included. Patients who were pregnant, allergic, having diabetes mellitus, on antibiotic therapy, or undergoing orthodontic therapy were excluded. A healthy control group consisting of 30 volunteers in the same age range was also included in the study. The study was approved

by the Ethics Committee of the Panjab University. The patients were informed of the study protocol, and written consent was obtained as per the Indian Council of Medical Education and Research guidelines.

The evaluation of periodontitis was on the basis of periodontal pocket depth and clinical attachment level (CAL): The number of erupted teeth were recorded, and a periodontal examination was performed on six sites of each standing tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) using a University of North Carolina-C-15 probe. The level of attachment was determined by subtracting from the depth of the pocket the distance from gingival margin to the cemento-enamel junction. The disease severity was described as being slight (mild) periodontitis (CAL $\leq 1-2$ mm), moderate periodontitis (CAL $\leq 3-4$ mm), or severe periodontitis (CAL ≥ 5 mm).^[15]

A questionnaire was administered for the details of patients' age, gender, smoking habits, presence or absence of clinical dental disease, and oral hygiene. The plaque index (Silness and Loe) for midbuccal and midlingual surfaces of all teeth was assessed, gingival index (Loe and Silness) was assessed by the periodontal examination. Kuppuswamy's modified version of the evaluation of socioeconomic status based on the score total of education, occupation, and income was followed.^[16] The stratification was done under five classes comprising upper, upper middle, lower-middle, upper-lower, and lower classes, respectively.

Sampling procedure

Sterile paper point was introduced into each periodontal pocket for 30–60 S. The paper point samples were pooled and transferred immediately to test tubes containing glucose-azide broth (Hi-Media laboratories, Mumbai) and taken to the laboratory for microbiological analysis.

Bacterial isolation and identification

The samples were inoculated onto the blood agar (Hi-Media Laboratories, Mumbai) plates and incubated in microaerobic condition. Every growth showing Gram-positive cocci, positive bile esculin, positive 6.5% NaCl tests, catalase negative, and biochemical tests was evaluated for the presence of enterococci as per standard procedure.^[17]

Statistical analysis

SPSS software version 16 (IBM Corporation, USA) was used for statistical analysis.

Age-wise matching with socioeconomic status, oral hygiene, smoking, and other variables was done by Pearson's Chi-square test. $P < 0.001$ was considered to be statistically significant.

RESULTS

A total of 100 individuals (males 52 and females 48) from different socioeconomic classes were evaluated for the prevalence of enterococci. The mean age of all the participants was 40.77 years. There was no significant difference for males and females. The dental awareness questionnaire [Table 1] revealed that frequency of cleaning teeth was significantly different in all socioeconomic classes. The regular attendance of dental clinic was also associated with the socioeconomic class. The upper middle class attended the dental clinic more regularly. The prevalence of enterococci was most in the lower class (100%), lower middle (70%), upper middle (70%), and the upper class decreasing to 31.25% [Table 2]. No enterococci was isolated from healthy control group from all socioeconomic classes. Enterococci were more prevalent (55%) in population who brushed the teeth for <2 min/day on an average than the population who brushed for >2 min/day on an average (45%) in all the socioeconomic classes [Figure 1]. Enterococci were more prevalent in smokers (88%) than nonsmokers (12%) among all

the socioeconomic classes. Enterococci were isolated in 64% of periodontitis patients who had fair-to-good level of plaque index [Figure 1] and 79% in patients who had mild-to-moderate clinical attachment loss (<4 mm). The level of enterococci was lower in mild periodontitis group 13/33 (39.4%), moderate periodontitis group 20/30 (66.67%), and in severe periodontitis group 6/7 (85.71%). The prevalence of enterococci was maximum in individuals with severe clinical attachment loss.

Of the 46 isolates, the species distribution of enterococci showed the predominant species was *E. faecalis* 39 (84.78%) followed by *E. faecium* 7 (15.21%). *Enterococcus durans* and other enterococci species were not isolated from any of the samples.

DISCUSSION

The study shows the prevalence of enterococci in the subgingival biofilm of periodontitis patients and its association with the socioeconomic group in an urban population. A significant positive correlation is seen in the presence of enterococci in periodontitis patients ($P < 0.001$) as compared to controls. A significant difference was also observed in the presence of enterococci with clinical parameters of CAL, probing depth and plaque accumulation ($P < 0.001$). There was a significant difference ($P < 0.001$) in the prevalence of enterococci in periodontitis patients who had fair-to-good plaque index. This also correlated with the socioeconomic groups, the lowest prevalence of enterococci in the upper class, and around 70% in the upper middle and lower middle classes and 100% in lower socioeconomic class. These results show the prevalence of opportunistic pathogen like enterococci is more in the lower strata. It could be due to poverty, lack of economic, and educational resources that oral hygiene awareness is deficient, and there is the existence of this social gradient.^[18] These oral health inequalities need to be addressed. Studies have shown that the difference in the oral health status of the

Table 1: Questionnaire

Name: _____	Age: _____	Gender: _____
Occupation: _____	Education: _____	
Income per month: _____		
Socioeconomic status (score on the basis of Kuppuswamy's scale): _____		
Chief complaint: _____		
History of present illness: _____		
Personal habits		
1) How many times do you brush your teeth? (once/twice/thrice/do not brush)		
2) How many minutes do you brush? (<2 min/ >2 min)		
3) Do you smoke yes/no		
If yes do you smoke less than/more than 20 cigarettes a day?		
If yes since how long have you been smoking?		
4) Plaque index-score		
Interpretation		

Table 2: Prevalence of Enterococci (%) in different urban-socioeconomic groups

Socioeconomic class (Kuppuswamy's scale)	Total samples (n)	Number of samples in which Enterococci isolated, n (%)	P
0<5 (lower class)	2	2 (100)	0.001
5-10 (upper-lower)	4	4 (100)	0.001
11-15 (lower-middle)	10	7 (70)	0.001
16-25 (upper-middle)	38	25 (69.4)	0.001
26-29 (upper)	16	5 (31.25)	0.001

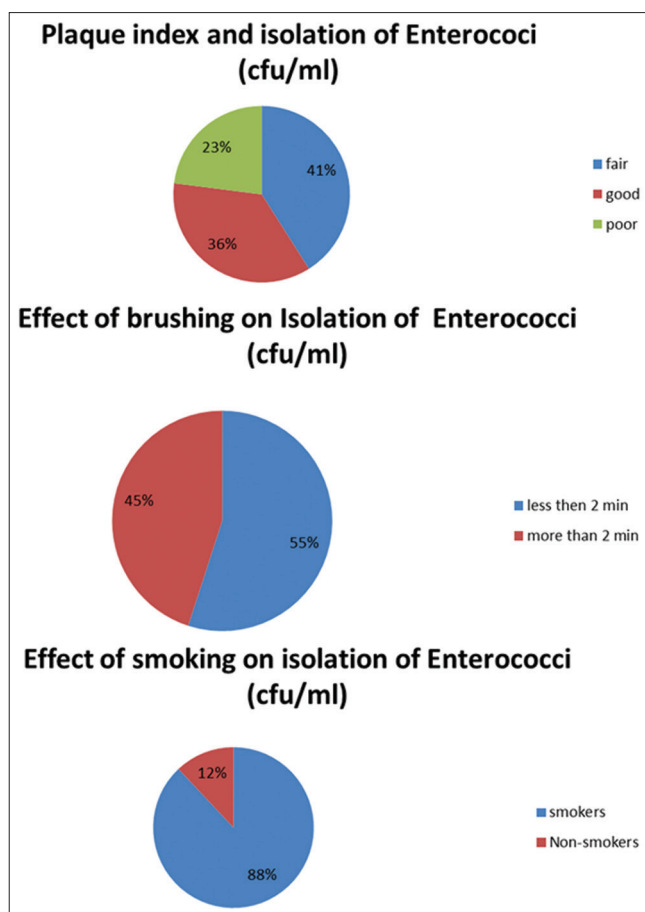


Figure 1: Prevalence of Enterococci (%) with relation to plaque index, frequency of brushing and smoking in all socioeconomic groups.

individuals of high economic status and those with the low economic status has increased over the last decade.^[19]

The present data show that periodontal infection may favor the colonization of the enterococci species. A higher prevalence of periodontitis and enterococci in lower socioeconomic group shows that the prevalence of periodontitis is inversely related to increasing family income and use of dental clinics.^[20] The presence of enterococci was found to be directly linked with periodontitis and smoking. In a recent study, it has been seen that smoking affects the subgingival bacterial profile in healthy individuals and is responsible for the depletion of beneficial bacteria and the increase in periodontal pathogenic bacteria.^[21] No enterococci was isolated from the control group healthy volunteers from all the socioeconomic groups. The origin of enterococci in oral cavity is still unclear. The gastrointestinal tract is the primary habitat for enterococci. Studies have proposed that enterococci enter the oral cavity, particularly the

antibiotic-resistant enterococci as contaminants. This antibiotic resistance could be laterally transferred to human commensals in the gut.^[22] This could be the reason no enterococci was reported in our study in control group, whereas periodontal infection may favor the colonization of this species. Recently, Vidana *et al.* have shown no foodborne transmission of enterococci.^[23] A study in Europe has reported a prevalence of oral enterococci as 1% in dental students and 6.6% in healthy individuals.^[24] Although studies in the west have reported an oral carriage of 16%–20% of enterococci in people having healthy and gingival and periodontal status as compared to 73% in people having gingivitis and periodontitis.^[25] In the human oral cavity, enterococci have been frequently detected in patients with periodontitis. enterococci are seen to be more prevalent with increasing severity of periodontitis. In our study, it was seen in 85.7% of individuals having severe periodontitis. This could be because of infection; the oral cavity may become a reservoir of the nosocomial enterococci which could further contribute to periodontal breakdown. enterococci carry virulence factors related to adhesion and biofilm formation colonizing different oral sites.^[26] Our study shows the predominant species as *E. faecalis* followed by *E. faecium*. Most of the studies have investigated *E. faecalis* species. Since *E. faecium* have emerged as important pathogen, particularly in nosocomial environment^[27] all the enterococci were identified. The survival ability of enterococci results in the bacterial persistence in subgingival biofilm after the periodontal treatment respectively.^[28,29] This indicates that enterococci play a crucial role in the severity or progression of periodontitis particularly in a favorable oral environment.

CONCLUSION

Enterococci are more prevalent in people whose oral hygiene is poor, socioeconomic status is low and who are smokers. Further longitudinal studies with large number of isolates are needed to know the oral enterococci distribution in various population groups. This silent reservoir of virulent enterococci may pose a threat of nosocomial infection.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or nonfinancial in this article.

REFERENCES

- Shaju JP, Zade RM, Das M. Prevalence of periodontitis in the Indian population: A literature review. *J Indian Soc Periodontol* 2011;15:29-34.
- Blas E, Kurup AS. Equity, Social Determinants and Public Health Programmes. Geneva: World Health Organization; 2010. p. 291.
- 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.
- Zoletti GO, Pereira EM, Schuenck RP, Teixeira LM, Siqueira JF Jr, dos Santos KR. Characterization of virulence factors and clonal diversity of *Enterococcus faecalis* isolates from treated dental root canals. *Res Microbiol* 2011;162:151-8.
- Balaei-Gajan E, Shirmohammadi A, Abashov R, Agazadeh M, Faramarzie M. Detection of *Enterococcus faecalis* in subgingival biofilm of patients with chronic refractory periodontitis. *Med Oral Patol Oral Cir Bucal* 2010;15:e667-70.
- Al-Badah Suliman AH, Ibrahim SS, Nasser A, Salamah AA, Shebl I, Salah S. Clonal diversity and antimicrobial resistance of *Enterococcus faecalis* isolated from endodontic infections. *Electron J Biotechnol* 2015;18:175-80.
- Mittal S, Singla P, Deep A, Bala K, Sikka R, Garg M, et al. Vancomycin and high level aminoglycoside resistance in *Enterococcus* spp. in a tertiary health care centre: A therapeutic concern. *J Pathog* 2016;2016:8262561.
- Kuch A, Willems RJ, Werner G, Coque TM, Hammerum AM, Sundsfjord A, et al. Insight into antimicrobial susceptibility and population structure of contemporary human *Enterococcus faecalis* isolates from Europe. *J Antimicrob Chemother* 2012;67:551-8.
- Sun J, Sundsfjord A, Song X. *Enterococcus faecalis* from patients with chronic periodontitis: Virulence and antimicrobial resistance traits and determinants. *Eur J Clin Microbiol Infect Dis* 2012;31:267-72.
- Chomicz L, Szubińska D, Piekarczyk J, Wojtowicz A, Piekarczyk B, Starościk B, et al. Occurrence of oral subclinical infections in insulin treated diabetics. *Wiad Parazytol* 2004;50:177-80.
- Mohammadzadeh Akhlaghi N, Rahimifard N, Moshari A, Vatanpour M, Darmiani S. The effect of size and taper of apical preparation in reducing intra-canal bacteria: A Quantitative SEM study. *Iran Endod J* 2014;9:61-5.
- Navabi AA, Khademi AA, Khabiri M, Zarean P, Zarean P. Comparative evaluation of *Enterococcus faecalis* counts in different tapers of rotary system and irrigation fluids: An *ex vivo* study. *Dent Res J (Isfahan)* 2018;15:173-9.
- Fabricius L, Dahlén G, Holm SE, Möller AJ. Influence of combinations of oral bacteria on periapical tissues of monkeys. *Scand J Dent Res* 1982;90:200-6.
- Kayaoglu G, Ørstavik D. Virulence factors of *Enterococcus faecalis*: Relationship to endodontic disease. *Crit Rev Oral Biol Med* 2004;15:308-20.
- Wiebe CB, Putnins EE. The periodontal disease classification system of the American Academy of Periodontology – An update. *J Can Dent Assoc* 2000;66:594-7.
- Kumar N, Gupta N, Kishore J. Kuppaswamy's socioeconomic scale: Updating income ranges for the year 2012. *Indian J Public Health* 2012;56:103-4.
- Fackland R, Sham DA, Teixeira LM. *Enterococcus*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH, editors. *Manual of Clinical Microbiology*. 7th ed. Washington: American Society of Microbiology; 1999. p. 297-5.
- Ahuja N, Ahuja N. Influence of Socioeconomic status and home environmental factors on oral health related quality of life among school children in North Bengaluru, India. A cross sectional study. *J Indian Assoc Public Health Dent* 2017;15:220-4.
- Axelsson P, Albandar JM, Rams TE. Prevention and control of periodontal diseases in developing and industrialized nations. *Periodontol* 2000 2002;29:235-46.
- Gundala R, Chava VK. Effect of lifestyle, education and socioeconomic status on periodontal health. *Contemp Clin Dent* 2010;1:23-6.
- Karaszeh JA, Al Habashneh RA, Marzouka NA, Thornhill MH. Effect of cigarette smoking on subgingival bacteria in healthy subjects and patients with chronic periodontitis. *BMC Oral Health* 2017;17:64.
- Miranda JM, Franco CM, Vázquez BI, Fente CA, Barros-Velázquez J, Cepeda A. Evaluation of Chromocult enterococci agar for the isolation and selective enumeration of *Enterococcus* spp. in broilers. *Lett Appl Microbiol* 2005;41:153-6.
- Vidana R, Rashid MU, Özenci V, Weintraub A, Lund B. The origin of endodontic *Enterococcus faecalis* explored by comparison of virulence factor patterns and antibiotic resistance to that of isolates from stool samples, blood cultures and food. *Int Endod J* 2016;49:343-51.
- Sedgley C, Buck G, Appelbe O. Prevalence of *Enterococcus faecalis* at multiple oral sites in endodontic patients using culture and PCR. *J Endod* 2006;32:104-9.
- Sedgley CM, Nagel AC, Shelburne CE, Clewell DB, Appelbe O, Molander A. Quantitative real-time PCR detection of oral *Enterococcus faecalis* in humans. *Arch Oral Biol* 2005;50:575-83.
- Al-Ahmad A, Müller N, Wiedmann-Al-Ahmad M, Sava I, Hübner J, Follo M, et al. Endodontic and salivary isolates of *Enterococcus faecalis* integrate into biofilm from human salivary bacteria cultivated *in vitro*. *J Endod* 2009;35:986-91.
- Franzetti L, Pompei M, Scarpellini M, Galli A. Phenotypic and genotypic characterization of *Enterococcus* spp. of different origins. *Curr Microbiol* 2004;49:255-60.
- Komiyama EY, Lepesqueur LS, Yassuda CG, Samaranyake LP, Parahitiyawa NB, Balducci I, et al. *Enterococcus* species in the oral cavity: Prevalence, virulence factors and antimicrobial susceptibility. *PLoS One* 2016;11:e0163001.
- Anderson AC, Jonas D, Huber I, Karygianni L, Wölber J, Hellwig E, et al. *Enterococcus faecalis* from food, clinical specimens, and oral sites: Prevalence of virulence factors in association with biofilm formation. *Front Microbiol* 2015;6:1534.