**Original Article**

**Subgingival prevalence rate of enteric rods in subjects with periodontal health and disease**

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**Abstract:**

**Background:** The prevalence of enteric rods and their association with chronic periodontitis has gained prominence recently. Although the prevalence of these organisms from the subgingival plaque sample was reported in the literature, the carriage rate of these rods in our population is lacking. The present study was undertaken to know the carriage rate of enteric rods from our population in patients with periodontal health and disease.

**Materials and Methods:** Eighty-four systemically healthy participants, inclusive of 46 males and 38 females, were selected for the study. The selected participants were subjected to a periodontal examination and were categorized into chronic periodontitis and healthy group. Subgingival plaque samples were taken from all the participants, plated onto McConkey agar plates, and incubated overnight at 37°C to check for the growth of organisms. The grown organisms were then cultured according to the standard procedures.

**Results:** Prevalence of 71% and 83% of enteric rods in subjects with periodontal health and disease, respectively, was found in our study which was statistically significant.

**Conclusion:** Although no significant differences exist in the prevalence of enteric rods between healthy and patients with chronic periodontitis, the prevalence rate of enteric rods in subgingival plaque samples is considerably high in our population.

**Key words:** Biofilm, chronic periodontitis, dental plaque, Enterobacteriaceae, oral hygiene, personal hygiene

**INTRODUCTION**

Enteric rods are a common term applied to a family of Gram-negative, facultatively anaerobic, nonsporing, nonacid-fast, straight rods inhabiting the intestine of humans. The members of the family are characterized by the production of an array of virulence factors, resulting in life-threatening infections such as septicemia, lower respiratory and urinary tract infections, and being resistant to commonly used antibiotics. The term enteric rods can be mainly applied to two families, namely Enterobacteriaceae and Pseudomonadaceae. Clinically significant members include the organisms belonging to the genera Escherichia, Enterobacter, Salmonella, Shigella, Citrobacter, Pseudomonas, Serratia, Hafnia, Proteus, Morganella, Yersinia, Providencia, and Edwardsiella.

The colonization of oral cavity with enteric rods such as Acinetobacter baumannii and Pseudomonas aeruginosa has gained importance recently. However, the exact role played by these organisms in the pathogenesis of periodontal disease and whether they are transient colonizers or part of the subgingival flora has a harmonious relationship with the periodontal microbes is unclear. These organisms are not unique to subgingival flora, as they can also be seen in other ecological niches found in the oral cavity such as tongue and tonsils. The distribution of these microorganisms in the subgingival dental plaque varies from one population to another depending on a multitude of factors such as geographic area, diet, and hygienic practices employed by the individuals.

The enteric rods gained prominence recently due to their ability to elaborate virulence factors implicated in causing nosocomial infections, capable of causing tissue destruction, and exhibiting a high synergistic relationship between periodontopathic organisms.

Studies have been done involving different population groups worldwide to know the...
prevalence of enteric rods. A study by Slots et al.[19] has shown the prevalence of these rods in an American population to be 14%, whereas the prevalence in Germany was found to be 13.5%.[15] In a Latin-American population,[14] a prevalence of 34% was seen whereas 27.9% was the prevalence in a Chinese population.[13] The above results denote that the prevalence is not uniform and varies with the population examined, economic status of the country, sampling technique employed, and the condition of periodontal apparatus. It can be seen that the data regarding the prevalence of enteric rods in our population are lacking. Hence, the primary objectives of the present study are to know the oral carriage rate of enteric rods in our population and secondarily to know whether there exists any difference in the prevalence rate of these organisms among individuals with healthy periodontium and with chronic periodontitis.

**MATERIALS AND METHODS**

**Subject selection**
This case–control study was done from July 2013 to August 2014 to find out the prevalence of enteric rods. All the participants reporting to the outpatient department were screened, and the participants who met the inclusion criteria were allowed to participate in the study. The study was performed according to the Declaration of Helsinki, as revised in 2000, and was approved by the Institutional Ethical Committee (IEC/TDCH/030/2014).

Eighty-four individuals, inclusive of 46 males and 38 females, were then selected to participate in the study. The study was well explained to all the participants in their regional language, and a written consent form was obtained from all the participants willing to participate in the study. The inclusion criteria to participate in this study include participants of either gender and a presence of at least twenty permanent teeth, excluding third molars. Exclusion criteria include smokers, pregnant women, history of any systemic diseases, and history of antibiotic usage or periodontal treatment within a period of past 6 months.

**Clinical examination**
All the selected individuals were subjected to periodontal screening done by a single examiner (ATR), which involved recording of probing periodontal pockets and attachment level (AL). The oral hygiene status, gingival health, and plaque scores were assessed by taking Oral Hygiene Index – simplified,[20] Silness and Løe gingival index,[21] and plaque index (Turesky Gilmore Glickman modification of Quigley-Hein plaque index),[22] respectively.

Periodontal pockets (PDs), using William’s periodontal probe, were measured as the distance from gingival margin to base of the pocket. PD for each tooth was measured on all the six surfaces (mesio- and distobuccal, mesio- and distolingual, midbuccal, and midlingual). They were then added to get a mean PD. AL was measured as the distance from the cement-enamel junction to the base of the sulcus using a periodontal probe. When the gingival margin was located either coronal to apical to cement-enamel junction, the distance between the cement-enamel junction and the gingival margin was added or subtracted from the pocket depth, respectively.

PD and clinical AL (CAL) were assessed using a probe with William’s marking in both the arches. All the six sites per tooth were assessed, and the measurements are calibrated to the nearest millimeter. Intraoral periapical radiographs were taken for all the patients with chronic periodontitis.

After examination, the individuals were divided into healthy controls ($n = 42$) and with chronic periodontitis ($n = 42$). Participants were considered to be healthy if the probing depth is $<3$ mm with no evidence of attachment loss and bleeding on probing. The diagnosis of chronic periodontitis was made based on the criteria defined by the American Association of Periodontology.[21]

**Sample collection**
All the selected individuals were subjected to plaque sample collection. Subgingival plaque samples were collected from pockets measuring 5 mm or more in patients with periodontitis. In patients with periodontal health, samples were collected from an incisor, premolar, and molar from all the quadrants. The plaque samples were collected with a sterile curette after removing the supragingival plaque and isolating the area with cotton pellets, in a test tube containing peptone water, labeled properly, and transported immediately to the Microbiology laboratory, Tagore Medical College for further processing.

The samples were processed immediately on arrival. One loopful (0.04 mm in diameter) of the specimen was inoculated onto MacConkey agar plates for the growth of enteric rods. The plates were incubated aerobically at 37°C for 1–2 days. Organisms, if grown, were Gram stained and characterized according to the colonial morphology. They were then speciated according to standard biochemical tests.[23] Species found on the MacConkey agar were enumerated as counts $\times 10^5$.

**Statistical analysis**
A statistical program SPSS version 22 (SPSS,Inc., Chicago, IL, USA). was used for all the statistical analyses. Descriptive statistics were calculated for all the variables. Kolmogorov–Smirnov test was applied to assess the goodness of fit to normal distribution. Unpaired Student $t$-test was used to find the differences in age and the clinical variables between the groups; Chi-square test was used to find an association between the presence or absence of enteric rods and periodontal parameters such as PD and CAL. Statistical significance was set at $P < 0.05$.

**RESULTS**
A total of 84 participants participated in the study, divided into healthy controls ($n = 42$) and subjects with chronic periodontitis ($n = 42$). The demographic details of the study population are shown in Table 1, and it can be seen that there was no significant gender difference between the groups. There was a statistically significant difference between the groups in all the clinical parameters studied. The participants in the control group have a probing depth of $<3$ mm, and a moderate grade of periodontitis was observed in periodontitis group. Smokers were totally excluded in both the groups.

Table 2 summarizes the distribution of enteric rods in the study and control group. A total of 66 strains were isolated
from the study population and four participants harbored two strains each. Four different genera were isolated from the periodontitis group and three genera were isolated from the healthy group. Enterobacter spp. was found only in the periodontitis group. The carriage rate of Gram-negative rods in the control and periodontitis group was 71.4% and 83.3%, respectively, and the total prevalence of these rods was found to be 77%. When the prevalence of enteric rods was compared between the groups, no statistically significant differences existed. Similarly, no statistically significant differences were seen for any of the organism, except Enterobacter spp. when the groups were compared.

Table 3 summarizes a correlation between the age groups and the presence of enteric rods. It can be seen no association between the presence of enteric rods and the age intervals studied.

Association between PD and the presence of enteric rods using Chi-square test is shown in Table 4. Healthy group had a pocket depth of <3 mm (mean of 0.96 ± 0.17) and the chronic periodontitis group had more than 3 mm (mean of 3.75 ± 0.79). No statistically significant differences exist with respect to the prevalence of enteric rods when the two groups were compared.

Table 5 summarizes an association between CAL and the presence of enteric rods in the chronic periodontitis group. While none of the healthy patients displayed attachment loss, the mean CAL in the chronic periodontitis group is divided into two groups: CAL with 0–1 mm and CAL with more than 1 mm. It can be seen that no association exists between the prevalence of enteric rods and CAL.

**DISCUSSION**

Enteric rods being a normal inhabitant of the human intestines have been detected in the subgingival biofilm with varied prevalence. Most of the data regarding the prevalence were contributed only by industrialized nations and the prevalence rate in those studies varied from 0.7% to 13.5%. Unfortunately, data from our population are lacking and hence as a first step we tried to find the oral prevalence of enteric rods in our population. Our study found a prevalence of 77% in participants in our population. This was considerably more than the prevalence rate found in the other studies. The differences seen can be attributed to the level of personal and oral hygiene practice followed, and microbial composition in our part of the country. The results clearly underscore the importance of maintaining personal hygiene and adequate disinfection protocol to be followed in dental practice to prevent cross infections.

Periodontitis is of multibacterial origin, and almost 12 putative periodontal pathogens have been identified. Recently,
Enteric rods are characterized by high pathogenic potential as they elaborate various enzymes which can degrade basement membrane, inactivate complement components, produce extracellular leukotoxins, and suppress lymphocyte proliferation. In addition, they are also highly tissue invasive. They have also been shown to persist after periodontal debridement and have been also implicated as a key pathogen in cases of refractory periodontitis. All these findings favor the hypothesis that enteric rods might be involved in the pathogenesis of periodontal disease.

These study results show that the periodontal pockets are populated with enteric rods, irrespective of periodontal health, in our study population. When the organisms were stratified according to the periodontal status in our study, patients with periodontitis tend to harbor more number of organisms than the healthy controls though the results were not statistically significant. This can be attributed to the technique employed to identify the prevalence of bacteria or less sample size in our study. It is to be remembered here, these rods can be temporary residents, as postulated by Martínez-Fabón et al., or might have a significant role in the periodontal disease pathogenesis.

Similarly, no statistically significant differences are seen when the clinical parameters such as probing depth or CAL is correlated with the presence of enteric rods. Since this is a preliminary report, further studies will be done in the future with a larger sample size.

The most frequently identified organism was Escherichia coli followed by P. aeruginosa in both the groups. Although statistically significant differences were not seen when the number of samples harboring these organisms was compared between the groups, the number of genera isolated from periodontitis patients were more than those isolated from the controls. The differences between the two groups would have been much appreciated if we would have subjected our samples to quantitative microbial identification techniques.

The clinical implications of these study results can be better understood only if the pathogenic potential of these organisms is identified. It is an established fact that the enteric rods isolated from other parts of our body possess a multitude of virulence factors and the same trend can also be seen with the enteric rods isolated from the oral cavity. A study by Goncalves et al. has shown that the enteric rods isolated from the subgingival plaque samples of periodontitis patients harbored multidrug-resistant and hydrolytic enzyme-producing strains which can get involved in tissue destruction and disease progression also attests this fact. The effect of these potential virulence factors produced by organisms such as A. actinomycetemcomitans can be accentuated when a favorable environment exists; the presence of synergistically working bacteria and a microenvironment favoring the growth of these organisms. Furthermore, the synergistic interactions between the proven periodontal pathogens and these enteric rods are not clear. Hence, future studies studying the presence of periodontal pathogens coexisting with these enteric rods might give a clear picture regarding the role of these organisms in the periodontal disease pathogenesis. Furthermore, the difference in the virulence potential of the strains isolated from the healthy and periodontitis patients must be identified.

Within the limitations of this study, it can be concluded that the carriage rate of enteric rods is high in our population and further studies need to be done to ascertain their role in the periodontal disease pathogenesis.

**CONCLUSION**

The carriage of enteric rods is considerably high with a prevalence of 77% in our population highlighting the importance of maintaining personal and oral hygiene. However, their role in the periodontal disease pathogenesis still remains unclear.

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**Conflicts of interest**

There are no conflicts of interest.
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