ORIGINAL ARTICLE

Oral carriage of enterobacteriaceae among school children with chronic nail-biting habit

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ABSTRACT

Context: Onychophagia or habitual nail-biting is widespread among children and adolescents, between 10 and 18 years. Prevalence estimates range from 30% during childhood to 45% in adolescence. Nail-biting habit can result in autoinoculation of pathogens and transmission of infection between body parts. **Aims:** The purpose of the study was to evaluate the differences in prevalence of Enterobacteriaceae (*E. coli* and *Enterobacter* spp) in saliva samples from subjects with and without chronic nail-biting habit. **Subjects and Methods:** One hundred and twenty-two subjects with chronic nail-biting habit and 122 subjects with no oral habit were enrolled in the study. All subjects were aged 11-15 years. The saliva samples were collected by oral rinse technique, samples were studied microbiologically. **Statistical Analysis Used:** Two-tailed Student's *t*-test and Chi-square/Fisher's exact test were used to find the significance of study parameters between the groups. **Results:** Enterobacteriaceae were detected in the saliva samples of 80 of the 122 nail-biting subjects, whereas Enterobacteriaceae were detected in the saliva samples of only 10 of the 122 subjects who were not nail-biters. This difference in prevalence was statistically significant ($P < 0.001$). **Conclusions:** Our results suggest a higher carriage of Enterobacteriaceae in the individuals having nail-biting habits when compared to individuals with no habits. Further studies need to be done to know the prevalence of Enterobacteriaceae species in different age groups.

Key words: Enterobacteriaceae, *E. coli*, nail-biting, orofecal contamination, saliva

INTRODUCTION

Oral cavity is a moist environment which is kept at a relatively constant temperature (34-36°C) and a pH close to neutrality in most areas, and thus supports the growth of a wide variety of microorganisms.[1] At birth, the oral cavity is sterile but rapidly is colonized by microorganisms present in the environment, particularly from the mother during the first feeding.[2] The naturally acquired microflora is essential for the normal development of the host physiology, and contributes to the host defenses by excluding exogenous microorganisms.[3] The oral microflora is highly diverse, and more than 500 species of bacteria have been isolated from the oral cavity.[4]

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The resident microflora of the mouth is distinct from that found elsewhere in the body owing to its unique biologic and physical properties.[5] Most of the microorganisms of other sites of the body will not colonize the mouth. Intestinal and skin microorganisms will regularly be introduced into the mouth, but they will not be able to compete successfully with the oral microorganisms for attachment sites and nutrients, and hence will be rapidly eliminated.[3]

Most of the oral microorganisms exhibit commensalism. Shift in bacterial community dynamics cause pathological changes within oral cavity and distant sites.[6] Complex interactions among microorganisms themselves and with the host tissues result in the formation of microbial biofilms on the hard and soft tissues of the oral cavity.[7] Oral health and disease depends on the interplay between the host and the oral microbial community.[7]

Oral diseases seem to appear after an imbalance among the indigenous microbiota, leading to the emergence of potentially pathogenic bacteria.[1] The wide range of microorganisms present in the oral cavity exist in harmony with each other and

the surrounding environment. This harmonious relationship can get altered depending on several factors like food habits, systemic diseases, debilitated conditions, immunological suppression, drugs, oral habits, etc.

Oral habits include digit sucking, pacifier sucking, lip sucking, lip biting, nail-biting, bruxism, self-injurious habits, mouth breathing, and tongue thrust.[8] These habits can act as carriers of numerous microorganisms from extra oral environment into the oral cavity. Of these many microorganisms, Enterobacteriaceae members are of interest as they are not commensals, but have been reported to be found in the oral cavity of individuals having these habits.[9]

Enterobacteriaceae is a family of aerobic and anaerobic gram negative bacteria that includes both nonpathogenic and pathogenic enteric microorganisms. The significant genera of this family are Escherichia, Klebsiella, *Enterobacter* spp, Proteus, Providencia, and Serratia; which are mainly distributed in soil, water, plants, and animals. They cause diseases such as meningitis, bacillary dysentery, typhoid, and food poisoning.[10]

Nail-biting is a common oral habit in children and young adults and most nail-biters bite all 10 fingers equally rather than selectively. Complications of nail-biting include damage to the cuticles and nails, self-inflicted gingival injuries, gingival swelling, and dental problems such as increased incisal wear and apical root resorption.[11] For children with inadequate and poor toilet hygiene, enteric bacteria can pose a potential threat by penetrating the body via the mouth as a result of nail-biting.[9]

E. coli and other enteric bacteria ingested through a chronic nail-biting habit could cause local and systemic infections. Therefore, determination of the prevalence of Enterobacteriaceae in the mouths of chronic nail-biters may be useful for clinicians.

The aim of the present study was to determine and compare the prevalence of Enterobacteriaceae in the saliva samples of Indian children with and without chronic nail-biting habit.

SUBJECTS AND METHODS

The study was a comparative cross-sectional microbiological study, conducted on the saliva samples of 122 chronic nail-biting subjects and 122 subjects without chronic nail-biting habit aged 11-15 years. Government schools in and around the city were selected. Children in the schools were screened and inducted into the study. Only the schools whose principal/head of the school permitted the students to take part in the study were included. Subjects who were uncooperative and/or apprehensive, who had other oral habits, who had recently suffered or were suffering from any medical illness, and who were presently on antibiotic therapy or using antiseptic mouthwash/rinse were excluded. Whole saliva samples were collected after recording the patient's details and oral findings.

A sterile sample collection bottle containing 10 ml of 0.1 M sterile phosphate buffered saline was given to each subject. Subjects were instructed to rinse their mouth for 60 s using sterile buffered saline and were asked to expectorate the rinse back into the containers labeled with patient details. Samples were transported to laboratory, where the samples were transferred into sterile test tubes, centrifuged, the supernatant was discarded, and deposit was resuspended in 1 ml of sterile phosphate buffered saline (PBS) to obtain a concentrated rinse. One loop full of concentrated rinse was inoculated on MacConkey agar culture media using standard streak plate method. The plates were inoculated at 37°C.

After 24 h, the culture plates were taken out from incubator and examined for growth. The lactose fermenting colonies appeared pink in color and non-lactose fermenting colonies appeared pale in color. The lactose fermenting colonies were picked using straight wire and smears were prepared, stained with Gram's stain and examined under microscope for gram negative bacilli. If gram negative bacilli were present, biochemical tests (indole, urease, citrate utilization, triple sugar iron agar, and motility test using mannitol motility media) were performed for identification of specific group of Enterobacteriaceae.

Results on continuous measurements were presented on mean ± standard deviation (SD; min − max) and results on categorical measurements were presented in number (%). Significance was assessed at 5% level of significance. Student's *t*-test (two tailed, independent) was used to find the significance of study parameters on continuous scale between two groups (intergroup analysis) on metric parameters. Chi-square/Fisher's exact test was used to find the significance of study parameters on categorical scale between two or more groups.

RESULTS

Out of the 122 subjects with nail-biting habit (study group) studied, 54.1% were males and 45.9% were females. Among the 122 subjects without any habits (control group), 54.9% were males and 45.1% were females. The mean age of the subjects in study group was 12.67 years (SD = ±1.23). The mean age of the subjects in control group was 12.62 years (SD = ±1.28).

Enterobacteriaceae were cultured from 65.6% (80) of the samples obtained from study group; but only in 8.2% (10) of the samples from control group. The difference in prevalence of Enterobacteriaceae among the study and control groups was highly significant with a $P < 0.001$ [Table 1].

E. coli was the most commonly isolated organism; isolated from salivary samples of 53.3% of study subjects and 7.4% of subjects in control group. *Enterobacter* spp was isolated from salivary samples of only 12.3% of study subjects and 0.8% of subjects in control group.

Prevalence of Enterobacteriaceae was highest among the study subjects aged 14 years. Isolation rate of Enterobacteriaceae was 64, 56.3, 67.6, 75, and 72.7% in study subjects aged 11, 12, 13, 14, and 15 years, respectively. Isolation rate of Enterobacteriaceae was 11.1, 14.3, and 6.3% in control subjects aged 11, 12, and 13 years, respectively. Whereas, Enterobacteriaceae were not isolated from any subjects aged 14 and 15 years in the control group [Table 2].

Enterobacteriaceae were isolated from 69.7% of the 66 male subjects in the study group and from 60.7% of the 56 female subjects. Enterobacteriaceae were isolated from 7.4% of the 68 male subjects in the control group, whereas Enterobacteriaceae were isolated from 9.3% of the 54 female subjects.

DISCUSSION

Natural oral microflora established during childhood changes throughout the stages of life under the influence of various environmental and behavioral factors.[12] Competition and antagonism mechanisms among resident oral bacteria may help to maintain the ecological balance by preventing the overgrowth of some resident bacterial species. There is a direct and dynamic relationship between environment and microflora, even at the microhabitat level.[13] If the profile of any microbial community is altered, pathogenic activity may be exhibited.[12] The balance of the oral microflora can shift due to changes in the diet, dentition, reduction in saliva flow, or parafunctional habits.[13]

Table 1: Enterobacteriaceae isolated in study and control groups

Bacteria	Controls (*n*=122)(%)	Study group (*n*=122)(%)	*P* value
Total	10 (8.2)	80 (65.6)	<0.001
E. coli	9 (7.4)	65 (53.3)	<0.001
Enterobacter spp	1 (0.8)	15 (12.3)	<0.001

Finger/nail-biting affects the tissues of the oral cavity in several ways. Dental attrition, root resorption, microfractures of the anterior teeth, gingival trauma, spread of dermatological infection into the oral cavity from diseases of the fingernail bed and localized malocclusions are potential oral or dental consequences of nail-biting.[14]

Chronic nail-biting can result in autoinoculation of pathogens and transmission of infection to other parts of the body. Pinworms are commonly found in school-age children. These children will have perianal irritation as a result of the infection. When children scratch to relieve the itching, Enterobacteriaceae, viruses and pinworm eggs lodge under their nails. The hand-to-mouth behavior in children who are fingernail-biters with inadequate or poor hand hygiene will result in spread of these microbes to oral cavity. Hence, microbial infections may be attributed to fecal-oral route of transmission.[10]

Enterobacteriaceae usually live in the intestinal tract. Up to 15% of the population may harbor Enterobacteriaceae in the oral cavity, mostly as transient commensals. Their oral carriage rate may increase in old age, and in conditions leading to reduced salivary flow.[10] For children with inadequate and poor toilet hygiene, enteric bacteria can pose a potential threat and danger by penetrating the body via the mouth as a result of nail-biting; so they can lead to various infections in the oral cavity.[9]

Hable *et al*., studied 490 Minnesota children and found 4.7% prevalence of Enterobacteriaceae.[15] Chang and Foltz (1960) in their study on 254 adult college students isolated coliform bacteria from oral cavity of 22 (8.6%) adults, of these, three were identified as *E. coli*.[15] Leitch *et al.*, in their study on Caucasians isolated coliforms from 5% of the dental plaque samples.[16] Mobbs *et al.*, showed a 6.6% prevalence of *Enterobacter* spp, Pseudomonadaceae, and *Acinetobacter* spp in 120 healthy individuals.[17]

Poeta *et al.*, (2009) did not recover Enterococci or *E. coli* from healthy volunteers, whereas 10 isolates were obtained from 19.5% of patients with fixed appliances.[3] Hagg *et al.*, (2004) evaluated the prevalence of Enterobacteriaceae in 13 males and 14 females during fixed orthodontic appliance therapy and found an increase in the Enterobacteriaceae

Table 2: Enterobacteriaceae isolated in different age groups

	Control group			Study group		
Age (years)	Number of patients	*E. coli* (%)	*Enterobacter* spp (%)	Number of patients	*E. coli* (%)	*Enterobacter* spp (%)
11	27	2 (7.4)	1 (3.7)	25	13 (52.0)	3 (12.0)
12	35	5 (14.3)	0	32	15 (46.9)	3 (9.4)
13	32	2 (6.3)	0	34	17 (50.0)	6 (17.6)
14	13	-	-	20	13 (65.0)	2 (10.0)
15	15	-	-	11	7 (63.6)	1 (9.1)
Total	122	9 (7.4)	1 (0.8)	122	65	15

prevalence after the initiation of therapy (25.9%) compared to baseline (11.1%).[18]

Studies have been done on oral carriage of Enterobacteriaceae in patients with systemic diseases. Back-Brito *et al.*, (2011) in their study on 45 human immunodeficiency virus (HIV) positive and 45 HIV negative patients detected a significantly higher number of Enterobacteriaceae and Pseudomonas in the oral cavities of the HIV positive patients compared to HIV negative patients.[19] *E. cloacae* was the most frequently isolated species in both groups. Similar types of studies have been performed on individuals suffering from conditions like HIV-1 (Schmidt-Westhausen 1991), and burning mouth syndrome (Samaranayake 1989).[20,21]

All these studies had shown an increased carriage of the Enterobacteriaceae in their salivary samples. A study on 56 stroke patients, by Zhu *et al.*, detected coliforms in 12 patients during the acute phase, whereas only one patient carried coliforms in the oral cavity 6 months after discharge from hospital.[22] Another study done by Samaranayake (1984) on coliform carriage in individuals undergoing cytotoxic therapy has revealed that, there was an increased carriage of the coliforms in this group. The possible reason for the increased carriage is said to be due to compromised immune status and an inability to maintain proper hygiene due to poor physical conditions.[23]

The present study was undertaken to test the hypothesis "Finger/nail-biting patients have an increased carriage of Enterobacteriaceae in the oral cavity due to spread by the fecal-oral route through contaminated hands or environmental objects".

A cohort of 244 school going children aged between 11 and 15 years was included in our study. Equal numbers of nail-biting and non-nail-biting students were included. Children who were ill, who had medical illness recently, who were on antibiotic therapy, who were using antiseptic mouthwash, and children with thumb sucking or other parafunctional habits were excluded to avoid false negative results and confounding bias.

Baydaş *et al.*, (2007) had reported a nail-biting prevalence of 45% among adolescents.[9] Assuming a prevalence of 45% among our population, at 95% confidence level and 20% allowable error, the sample size was calculated as 244. Shetty and Munshi (1998) in their epidemiological study on 4,590 school children found that prevalence of nail-biting (12.7%) was more common in 13-16 years age group.[24] Leung and Robson (1990), in their review on nail-biting habit found that 45% of adolescents were nail-biters, whereas only 28-33% of children between the ages of 7 and 10 years were nail-biters.[11] Based on the findings of these authors, subjects aged between 11 and 15 years were included in the study population.

Samaranayake *et al.*, (1986) found that sensitivity of impression culture, the neat rinse culture (NRC), and the concentrated rinse culture (CRC) were 94%, 88% and 92% respectively. They found that the CRC was simple to perform; equally sensitive; and superior in quantifying yeast, coliform, and *S. aureus* carriage than the imprint culture technique.[25] Therefore it was, suggested that the CRC technique be preferentially employed in future investigations. Hence, oral rinse technique, described by Samaranayake *et al.*, (1986), was followed for sample collection.

The Enterobacteriaceae are usually considered to be only transiently present in the human oral cavity, but oral structures are still known to become infected with them, although other sites of the body are much more frequently involved.[9]

Gingival injuries like recession, ulceration, localized gingival swelling, and infection have been reported by Creath *et al.*, (1995)[14], Ware (1980) and Hodges *et al.*, (1994).[26,27] But in our cohort of 244 patients localized gingival injuries attributed to finger nail-biting were not present.[24]

Among the clinical conditions of oral structures involving enteric bacteria, there have been reports of post-extraction cellulitis caused by *Salmonella choleraesuis*, infection of maxillary epithelial cyst caused by *Salmonella typhimurium*, and osteomyelitis of the mandible caused by a mixed flora with *E. aerogenes and E. coli.* In the present study we did not come across any such conditions.

In a study, Baydaş *et al.*, (2007) evaluated the effect of chronic nail-biting on oral carriage of Enterobacteriaceae. Their study group consisted of 25 subjects having chronic finger/nail-biting habit and 34 subjects in the control group. The present study was conducted on larger cohort of 122 nail-biting subjects and 122 controls.[9]

The age of the patients showing nail-biting ranged from 11.9 to 16.5 years, the age of the patients with no oral habit ranged from 12.4 to 16.9 years. In the present study all our patients were between 11 and 15 years of age. The mean age of the subjects in the study group was 12.62 ± 1.28.

In the study by Baydaş *et al.*, the study and control groups were not gender matched. There were 8 boys and 17 girls in the study group; whereas control group consisted of 16 boys and 18 girls. In the present study similar number of female subjects was included in both study group 55 (45.1%) and control groups 56 (45.9%).[9]

In the present study, Enterobacteriaceae were isolated in 80 of the 122 nail-biting subjects; whereas Enterobacteriaceae were isolated in only 10 of the 122 control subjects. On the other hand, *E. coli* was detected in 65 (53.3%) subjects with chronic nail-biting while *E. coli* was isolated in nine subjects with no oral habit. There was a statistically significant

difference ($P < 0.001$) in the prevalence of Enterobacteriaceae between these two groups. In our study the prevalence of Enterobacteriaceae when compared between the nail-biting and control group revealed a higher carriage among the nail-biters (65.6%), than controls (8.1%). These findings are consistent with the findings of Baydaş *et al.*, who also found a higher carriage of Enterobacteriaceae among nail-biters (76%), than control group (26.5%).[9]

The findings of the present study and that of Baydaş *et al.*, indicate that there is definitely a higher prevalence of Enterobacteriaceae among nail-biting individuals than the individuals without any habit.[9]

This higher prevalence of Enterobacteriaceae among subjects with nail-biting could be due to orofecal route of transmission of Enterobacteriaceae and poor general hygiene maintenance.

Further studies need to be done to know the exact prevalence of the Enterobacteriaceae species in different age groups and to rule out if there is any association between oral hygiene status and prevalence of Enterobacteriaceae. In addition, association between the oral and perianal prevalence of Enterobacteriaceae and systemic complications following autoinoculation through the habits also needs to be evaluated.

It is important to recognize and eliminate nail-biting habit with a proper counseling and appropriate treatment. Individuals with chronic nail-biting habit should be given proper hygiene instructions to prevent contamination.

Dental specialists should take into consideration the increase in prevalence of Enterobacteriaceae in patients with nail-biting and whenever required, should seek consultation with the patient's medical specialist, because any surgical intervention in an individual having these habits are prone for the further systemic complications which could prove to be of medical significance. Individuals with chronic nail-biting habits should be given proper hygiene instructions to prevent contamination, and proper counseling towards the elimination of nail-biting habit.

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