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Influence Of Habit Intervention Appliance On The Oral Carriage Of *Enterobacteriaceae*

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Abstract:
Background: *Enterobacteriaceae are human intestinal bacteria that are also found in soil, plants and animals. An oral carriage of these bacteria is a common occurrence following thumb sucking and/or nail-biting habits, resulting in local and systemic infection.*
Objective*: To study the effect of habit intervention appliance on oral inoculation of Enterobacteriaceae in chronic thumb sucking and nail-biting habits. The antibiotic susceptibility of the microbes was also investigated.*
Materials and Methods*: Sixty-four healthy co-operative children with chronic thumb-sucking and nail-biting habits were enrolled. The saliva and swab samples were streaked using an inoculating loop on MacConkey agar and studied microbiologically before and after habit intervention. Gram staining and biochemical tests were conducted. Antibiotic susceptibility of microbes was performed by disk diffusion assay using Mueller Hinton agar medium.*
Results*: Enterobacteriaceae was present in all children. The oral carriage of E. coli was significantly more than Klebsiella in both habits. At baseline, E. coli and Klebsiella were 81.3% (52/64) and 18.8% (12/64) respectively which after habit intervention, reduced to 29.7% (19/64) and 3.1% (2/64) respectively and the results were statistically significant (p-value <0.001). 87.5% of the bacterial samples demonstrated sensitivity to amoxicillin. Cephalexin was the most resistant of all antibiotics (62.5%).*
Conclusion*: Enterobacteriaceae was evident in nail-biting and thumb sucking habits. A statistically significant reduction in the oral carriage of E. coli and Klebsiella was observed after habit intervention appliance, emphasizing its importance in minimizing and/or preventing the spread of infection. Both microbes were most sensitive to amoxicillin and were resistant to cephalexin.*
Keywords: *child; Enterobacteriaceae; habits; nail biting; prevalence; saliva; thumb sucking*

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I. Introduction

The natural oral microbiota that develops in childhood continuously evolves during life[1]. The delicate equilibrium of oral microflora is affected by changes in the environment such as diet[1], dentition[2], oral hygiene[1,3], and saliva[4]. A shift in the microbial community profile indicates a pathogenic activity. The transition of normal flora to a pathogenic form in the oral cavity is a predisposing and/or aggravating factor for many oral and systemic diseases[5]. One of the common modes of disruption of oral flora in children is through parafunctional habits such as nail-biting and thumb sucking wherein an autoinoculation of microbes often occurs by members of the *Enterobacteriaceae* family[6].

Enterobacteriaceae is an extensive microbial family consisting of Gram-negative, facultative anaerobes with multiple genera and species[7]. Among the genera, the most prominent ones are *Escherichia coli (E. coli)* and *Klebsiella.* These gut bacteria, which also exist in in soil, water and food, pose significant health risks when introduced into the oral cavity through contaminated foods, improper hand hygiene practices coupled with thumb sucking and nail-biting habits increase the oral carriage of these microbes. Since oral cavity is the gateway to the body, debilitating systemic conditions such as endocarditis, pneumonia, septicaemia, and meningitis can occur (8), necessitating antibiotic use. Chronic habits like thumb sucking and nail-biting facilitate their transmission, making early intervention critical.

Nail biting or onychophagia occurs in 28-33% of children between the ages of 7-10 years[9]. Although rare in children below 3 years, the prevalence increases between the ages of 3-6 years[10] and peaks thereafter till adolescence, approximating 45% in the pubertal period[9]. Thumb-sucking habit ranges from 30-40% among preschoolers[11] to 50% in all children[12].

Timely intervention of such habits is essential to minimize the detrimental effects of these microbes on local and systemic health. Counselling, behavioural modification techniques, stimulus control therapy, habit reversal training, habit-breaking appliances, and pharmacotherapy are some of the methods employed for the interception of habits[13]. Among the habit-breaking appliances, pre-orthodontic trainers have gained popularity because of their removable, light, and flexible features. The prefabricated myofunctional appliance trains the muscles, restores balance and function, guides tooth eruption, and encourages tooth alignment[14].

The present study aimed to investigate the influence of habit intervention appliance on the oral carriage of *Enterobacteriaceae* among children with chronic thumb sucking and nail-biting habits. The study also aimed to record the species susceptibility of these microbes to various antibiotics.

II. Materials And Methods

Study Design: One-group pretest-posttest quasi-experiment study was designed.

Ethical Approval: The cross-sectional study was approved by Panjab University Institutional Ethical Committee (PUIEC201210149/A-1/01/03).

Study Duration: The study was conducted between January to December 2021

Sample size: Sixty-four children

Sample size calculation: The convenience sampling method was used because of the vicinity, accessibility and feasibility of the trial during pandemic times. The sample size was estimated based on the study by Kamal and Bernard (α=0.05 and β=0.2)[6].

Inclusion Criteria: Co-operative and healthy children, aged 5-10 years, with chronic thumb sucking and/or nail-biting habits were recruited in the trial.

Exclusion Criteria: Children with systemic disorders, infection, cleft lip/palate, skeletal jaw discrepancies, trauma/surgery in the orofacial region, undergoing orthodontic intervention, using mouthwash/antibiotics, and uncooperative patients were excluded.

Procedure methodology:

After written informed consent was obtained, the patient's demographic details, oral hygiene practices, thumb sucking and nail-biting habits were recorded. A general physical examination along with complete extra-oral and intra-oral examination was conducted.

The children were asked to report in the morning with instructions to refrain from eating or drinking at least 30 minutes before sample collection to avoid contamination. They were made to sit with the head tilted forward to facilitate spitting of saliva. Five ml of unstimulated saliva was collected in a sterile wide-mouth container and closed with a tight lid to prevent leakage or contamination. The container was clearly labelled and immediately transported to the microbiology laboratory of the Institute for processing and identification.

A sterile swab was moistened with normal saline and gently rubbed on the pre-identified area on the digit/nail with light pressure. The swab was used in a rotating motion to maximize contact with the skin. A moistened swab is known to improve collection efficiency by increasing the adherence of microorganisms to the swab. It was then, placed in a labelled, sterile collection tube containing normal saline and transported to the microbiology laboratory for processing and identification. Transportation of the swab in normal saline prevents desiccation of the sample and maintains viability.

Saliva and swab samples were collected and handled following aseptic procedures. Both samples were streaked using an inoculating loop on MacConkey agar and incubated at 37º C for 24-48 hours. Gram staining was performed from the colonies obtained on the MacConkey agar plate (Figure 1a-1b) and biochemical testing was done for identification of bacteria. Confirmatory biochemical tests such as indole, citrate, urease, and TSI (Triple Sugar Iron) agar test were conducted for the final identification of bacterial isolates (Figures 2a-2d).

Among the salivary *Enterobacteriaceae*, *E. coli* tested positive for the indole test by forming a red-coloured ring near the surface of the medium whereas citrate and urease tests were negative. *Klebsiella* showed a positive urease test by forming a pink colour and a positive citrate test by turning the slant into a blue colour whereas indole test was negative. For the TSI agar test, both *E. coli* and *Klebsiella* showed fermentation of all three sugars (glucose, lactose, and sucrose).

Additional testing for antibiotic susceptibility of *E. coli* and *Klebsiella*, isolated from saliva and swab samples of children, was performed using disk diffusion assay following the Clinical and Laboratory Standard Institute guidelines. The effect of amoxicillin, ampicillin, azithromycin, cephalexin, and ceftriaxone on the isolated bacteria was evaluated. Mueller Hinton agar medium was used for the disk diffusion test (Figure 3). Based on the zone of inhibition measurements, the isolates were classified as “susceptible,” “intermediate,” or “resistant”.

The subjects were made to wear a prefabricated orthodontic trainer appliance T4K-First Phase (Pre-Orthodontic Trainer, Myofunctional Research Co., Queensland, Australia) and were duly instructed about its use and cleanliness (Figure 4). The initial advisory was to wear the device for 15 minutes a day, then gradually increase the duration of wear to 1 hour each in the morning, afternoon, and evening hours. These hours were specifically chosen to coincide with the timings of stressful activities. The children with thumb-sucking habits were further instructed to wear the appliance overnight. On the follow-up visit after 6 months, the same procedure of saliva and swab collection was followed, and the specimens were sent for microbiological and biochemical analyses as described above.

Statistical analysis

The data was statistically analysed using SPSS version 22.0 (IBM SPSS Statistics Inc., Chicago, Illinois, USA). Categorical variables were reported as counts and percentages. Group comparisons were made with the Chi-Square test if all expected cell frequencies were more than 5 and Fisher’s Exact test when expected cell frequencies were less than 5. McNemar test was conducted for comparison between categorical values of pre- and post-intervention (comparison of variables of baseline data with FU variables). p-value < 0.05 was considered significant.

III. Results

In Table 1, of the 64 subjects studied, 48.4% were males and 51.6% were females. Most subjects were in the nail-biting group (46.9%) followed by the thumb sucking group (32.8%) and the combined group (20.3%). The prevalence of both habits was significantly higher in the 7-9 year-age group. However, there was no significant gender difference.

In this study *Enterobacteriaceae* were observed in all children indulging in nail-biting and/or thumb-sucking habits. *E. coli* was significantly more (81.3%-52/64) than *Klebsiella* (18.8%-12/64-Table 1) in both males and females and in the 7–9-year age group.

Among the habits (Table 2), *E. coli* was highest in the combined group (92.3%-12/13) followed by the nail-biting group (86.7%-26/30) and the thumb sucking group (66.7-14/21*Klebsiella* was predominant in the thumb sucking group (33.3%-7/21) followed by the nail-biting group (13.3%-4/30) and the combined group (7.7%-1/13). These findings indicating a trend that highlights the need for habit intervention.

The oral carriage of salivary *Enterobacter* (*E. coli* and *Klebsiella*) reduced from 100% (64/64) in the pre-intervention period to 32.8% (21/64) in the post-intervention period and the result was statistically significant (p-value <0.001-Table 2). Among these microbes, *E. coli* reduced from 81.3%

(52/64) to 29.7% (19/64) respectively and *Klebsiella* from 18.8% (12/64) to 3.1% (2/64) respectively after habit intervention appliance. The results were statistically significant (p-value < 0.001). However, swab samples on the thumb/nail showed bacterial growth in only 20.3% subjects (13/64) in the pre-intervention period which reduced to 12.5% (8/64) in the post-intervention phase.

The antimicrobial susceptibility tests showed that most bacterial isolates were susceptible to amoxicillin, ampicillin, azithromycin, and ceftriaxone. Overall, amoxicillin was the most effective antibiotic against these Gram-negative bacteria, with 87.5% of the samples demonstrating sensitivity to this drug. The least effective antibiotic was cephalexin, with only 14.1% of the samples demonstrating sensitivity to this drug which was also the most resistant of all antibiotics (62.5%)- Table 3.

IV. Discussion

Children are particularly vulnerable to faeco-oral contamination because of inadequate knowledge and awareness about hygiene, improper toilet, and hand hygiene practices, frequent contact with contaminated soil, ingestion of contaminated food and water, and low immune systems. Gut microbes have a symbiotic relationship with the host in healthy individuals[15]. However, these microbes enter the oral cavity through chronic digit sucking and nail-biting habits, commonly observed in children. Such habits are detrimental to the dentofacial complex resulting in infections and a host of other adverse systemic consequences. Our study's use of microbiological techniques to isolate and analyse *Enterobacteriaceae* provides valuable insights into the prevalence and behaviour of these pathogens. Continuous surveillance and research are vital to monitor resistance patterns and develop effective treatments.

The present study observed no statistically significant gender difference pertaining to both habits. Similar observations on nail-biting habit were observed by Ghanizadeh et al in 743 nail-biting primary school children[16]. Malone and Massler[17] also reported comparable findings till 10 years of age, but an increase of 10% in boys thereafter. The reason for insignificant gender predilection could be the active participation of boys and girls in sports and academics respectively, both of which are competitive and stress-inducing. On the contrary, Kharbanda et al[18] observed that thumb sucking was significantly more in girls (1.0%) as compared to boys (0.4%) among 5554 Delhi children, aged 5-13 years. The present study observed no gender predilection, possibly because of its common aetiology which often follows imitation, psychological issues, and feelings of pleasure or insecurity.

In the present study, both nail-biting and thumb-sucking habits predominated the older 7–9-year age group. This could be attributed to pandemic times when health issues, loss of loved ones, financial loss, child abuse, and isolation occurred, resulting in feelings of loneliness (away from school and friends), sibling imitation, stress and/or ability to relate more to parental feelings of insecurity.

The present study observed that *Enterobacteriaceae* was present in all children. The oral carriage of salivary *Enterobacteria* was most in the nail-biting group (46.9%) followed by the thumb sucking group (32.8%) and the combined group (20.3%). The study is in accordance with the findings of Kamal and Bernard[6] who found 42% cases of salivary *Enterobacteri*a in the nail-biting group compared to 30% in the thumb sucking group and 10% in the control group of 8-15-year-old children. However, Baydas et al[19] observed still higher carriage of salivary *Enterobacteria* (76%-19/25) in the nail-biting group compared to the non-nail-biting controls (26.5%-9/34) in children with a mean age of 13.5±1.9 years which they had attributed to poor oral and general hygiene.

Biochemical analysis was conducted on isolated bacterial colonies to determine the oral carriage of *E. coli* and *Klebsiella* using indole, citrate, urease, and TSI agar tests. Comparable techniques of isolation were employed by Reddy et al[20] for the isolation of specific *Enterobacteriaceae* bacteria.

The present study noted that among all isolated salivary *Enterobacter*, 81.3% were *E. coli*, of which 92.3% were in the combined group followed by 86.7% in the nail-biting group and 66.7% in the thumb sucking group. A similar study done by Reddy et al[20] showed a significantly higher (p < 0.001) carriage of salivary *Enterobacteria* among nails biters (65.6%) compared to controls (8.2%) with *E. coli* being the most common (53.3%) isolated microbe. The study also mirrors the findings of Kamal and Bernard[6] wherein saliva samples of 8–15-year-old children showed a higher carriage of *Enterobacteriaceae* (42%), with more *E. coli* in the nail biters group (58.8% or 10/17). However, their thumb sucking group showed a predominance of *Klebsiella* (41.7% or 5/12). Similar findings were reported in the present study where *E. coli* predominated all groups, but a higher oral carriage of *Klebsiella* was observed in the thumb sucking group (33.3%) compared to the nail-biting group

(13.3%) and the combined group (7.7%). An increased oral carriage of *Klebsiella* in the latter group may be the mode of transmission through person-to-person contact, contact of contaminated equipment/surfaces, or wounds, all of which involve frequent contact by hand/touch.

Chinnasamy et al[21] observed that girls had higher *E. coli* because of frequent indulgence in the habit due to their more sensitive and expressive nature. However, the present study observed no gender predilection (p-value 0.447). But significantly more *E. coli* was observed in the 7–9-year age group. This can be because of the frequent performance of habits in this age group, possibly because of increased academic pressure, imitation, tackling fear and coping with challenges in pandemic times. It is thus, essential to implement educational programs in schools and communities to teach children about proper hygiene practices and the risks associated with nail-biting and thumb sucking. Enhancing such awareness can play a crucial role in preventing initial microbial colonization and reducing infection risks. Additionally, providing psychological support and counselling for children can help reduce stress-induced habits.

To discourage oral habits and restrict oral microbial infection, the present study used a prefabricated T4K habit breaking appliance. Instructions on cleanliness and non-sharing of the appliance were provided. The appliance, being a mechanical barrier, prevents access of digit into the mouth, assists in elimination of habit, and restricts the entry of microbes. It is prefabricated, flexible, less bulky and encourages patient compliance. The present study observed a statistically significant reduction in the oral carriage of *Enterobacteriaceae* (*E. coli* and *Klebsiella*) from 100% to 32.8% ($p < 0.001$) with appliance use. Studies have consistently proved that an appliance, particularly the fixed one, encourages plaque deposition and harbours bacterial growth, especially *Enterobacteriaceae* colonization. Hagg et al[22] in his study used oral rinse, pooled plaque, and imprint culture methods to evaluate the prevalence of *Enterobacteriaceae* during fixed orthodontic therapy and observed an increase in the prevalence of *Enterobacteriaceae* after initiation of therapy (25.9%) as compared to baseline (11.1%). Though removable, the T4K appliance has an added advantage in that it favours tooth alignment, assists in correction of malocclusion, and hence, minimizes plaque accumulation and microbial colonization. Furthermore, regular dental check-ups and microbial monitoring can help identify and address any re-emergence of these habits or infections early.

E. coli and *Klebsiella* are notorious for causing both community and hospital-acquired infections, which can lead to life-threatening diseases such as genito-renal infections, septicaemia, meningitis and pneumonia[23]. These infections pose a significant public health challenge due to the microbes' ability to produce extended spectrum beta-lactamase (ESBL) enzymes, which confer resistance to multiple antibiotics[24]. Our findings are particularly relevant in South-East Asia, especially India, where the high population density, ease of availability of substandard drugs, and unregulated antibiotic use exacerbate antibiotic resistance[25]. Such blood-stream infections often extend for prolonged periods, causing increased fatality rates, more so in children because of low immunity. The emergence of antibiotic-resistant bacteria is a critical public health issue worldwide. Our study adds to the growing body of evidence that underscores the importance of targeted interventions to curb the spread of resistant pathogens. The high resistance levels observed in *Enterobacteriaceae* align with global concerns about the misuse and overuse of antibiotics, which contribute to the development of resistant strains.

Our study revealed a significant reduction in the oral carriage of these microbes following habit intervention appliance. This finding emphasizes the urgent need for effective habit interventions to minimize microbial carriage and spread of infections. However, our results also highlighted high levels of antibiotic resistance among these pathogens, particularly their resistance to cephalexin (62.5%), while they remained sensitive to amoxicillin (87.5%). To address the rise in antibiotic-resistant infections, comprehensive strategies are essential. These include promoting personal and oral hygiene to prevent initial colonization and implementing stringent regulations on the sale and use of antibiotics to prevent misuse and overuse. Continuous surveillance and research are necessary to monitor resistance patterns and develop effective treatments. Public health interventions should encourage the use of mechanical barriers, such as the T4K habit-breaking appliance, which our study found to be highly effective in reducing microbial entry and colonization. By adopting these targeted strategies and promoting prudent antibiotic use, we can significantly improve public health outcomes and reduce the risk of infection spread.

V. Conclusion

The oral carriage of *Enterobacteriaceae* was evident in both nail-biting and thumb-sucking habits. *E. coli* was significantly more than *Klebsiella* in both genders and predominated the 7–9-year age group. A statistically significant reduction in the oral carriage of these microbes was observed after habit interception. The study highlights the dual function of myofunctional appliance in reducing microbial load, through restriction of microbes and correction of malocclusion. Additionally, it is crucial for practitioners to implement educational programs that raise awareness about proper hygiene practices related to thumb sucking and nail-biting to further reduce infection risks. Our study not only addresses a local health concern but also aligns with global efforts to combat antibiotic resistance. By implementing targeted interventions and promoting prudent antibiotic use, we can mitigate the threat posed by resistant pathogens and improve public health outcomes.

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Table 1: Distribution of sample n (%)

Distribution n=64			Habit				Microbe		
			TS 21 (32.8%)	**NB 30 (46.9%)**	**TS+NB 13 (20.3%)**	**p-value**	***E.coli* 52 (81.3)**	***Klebsiella* 12 (18.8)**	**p-value**
Gender	**Males**	31 (48.4)	10 (32.3)	15 48.4)	6 (19.4)	0.9704	24 (77.4)	7 (22.6)	0.002*
	Females	33 (51.6)	11 (33.3)	15 45.5)	7 (21.2)		28 (84.8)	5 (15.2)	<0.001*
Age in years	**5**	6 (9.4)	4 (19.0)	2 (6.7)	0	0.008**	3 (5.8)	3 (25.0)	1.000
	6	6 (9.4)	2 (9.5)	4 (13.3)	0		4 (7.7)	2 (16.7)	0.414
	7	11 (17.2)	1 (4.8)	3 (10.0)	7 (53.8)		9 (17.3)	2 (16.7)	0.035*
	8	19 (29.7)	9 (42.9)	9 (30.0)	1 (7.7)		16 (30.8)	3 (25)	0.003*
	9	20 (31.3)	5 (23.8)	11 36.7)	4 (30.8)		18 (34.6)	2 (16.7)	<.001*
	10	2 (3.1)	0	1 (3.3)	1 (7.7)		2 (3.8)	0	-

TS: Thumb sucking; NB: Nail-biting

Table 2. A comparison of the oral carriage of *E. coli* (n=52) and *Klebsiella* (n=12) pre- and post-habit intervention

Habit	Microbes	Period			p-value
		Pre-intervention n (%)		Post intervention n (%)	
Thumb sucking 21	*E. coli*	14 (66.7)		6 (28.6)	0.008**
	Klebsiella	7 (33.3)		0	0.031*
	Total in TS	**21 (100.0)**		**6 (28.6)**	**0.0011****
Nail biting 30	*E. coli*	26 (86.7)		10 (33.3)	<0.001**
	Klebsiella	4 (13.3)		2 (6.7)	0.500
	Total in NB	**30 (100.0)**		**12 (40.0)**	**0.0007****
Thumb sucking + nail biting 13	*E. coli*	12 (92.3)		3 (23.1)	0.004**
	Klebsiella	1 (7.7)		0	1.000
	Total in TS+ NB	**13 (100.0)**		**3 (23.1)**	**0.007****
	Cumulative microbes	*E. coli*	**52 (81.3)**	**19 (29.7)**	**<0.001****
		Klebsiella	**12 (18.8)**	**2 (3.1)**	**<0.001****
		Total	**64 (100.0)**	**21 (32.8)**	**<0.001****

Table 3. Antimicrobial susceptibility of *E. coli* and *Klebsiella*

Antibiotic	Sensitive	Intermediate	Resistant
Amoxycillin	56 (87.5%)	4 (6.3%)	4 (6.3%)
Ampicillin	42 (65.6%)	12 (18.8%)	10 (15.6%)
Azithromycin	42 (65.6%)	9 (14.1%)	13 (20.3%)
Ceftriaxone	42 (65.6%)	9 (14.1%)	13 (20.3%)
Cephalexin	9 (14.1%)	15 (23.4%)	40 (62.5%)
p-value	**<0.001****		

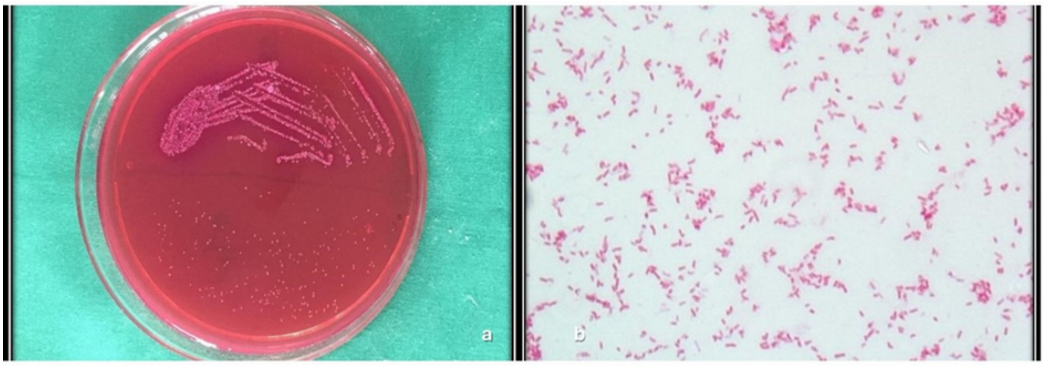

Figure 1a-1b – Colonies obtained on the MacConkey agar plate; Photomicrograph revealing Gram staining of *Enterobacteriaceae* colonies

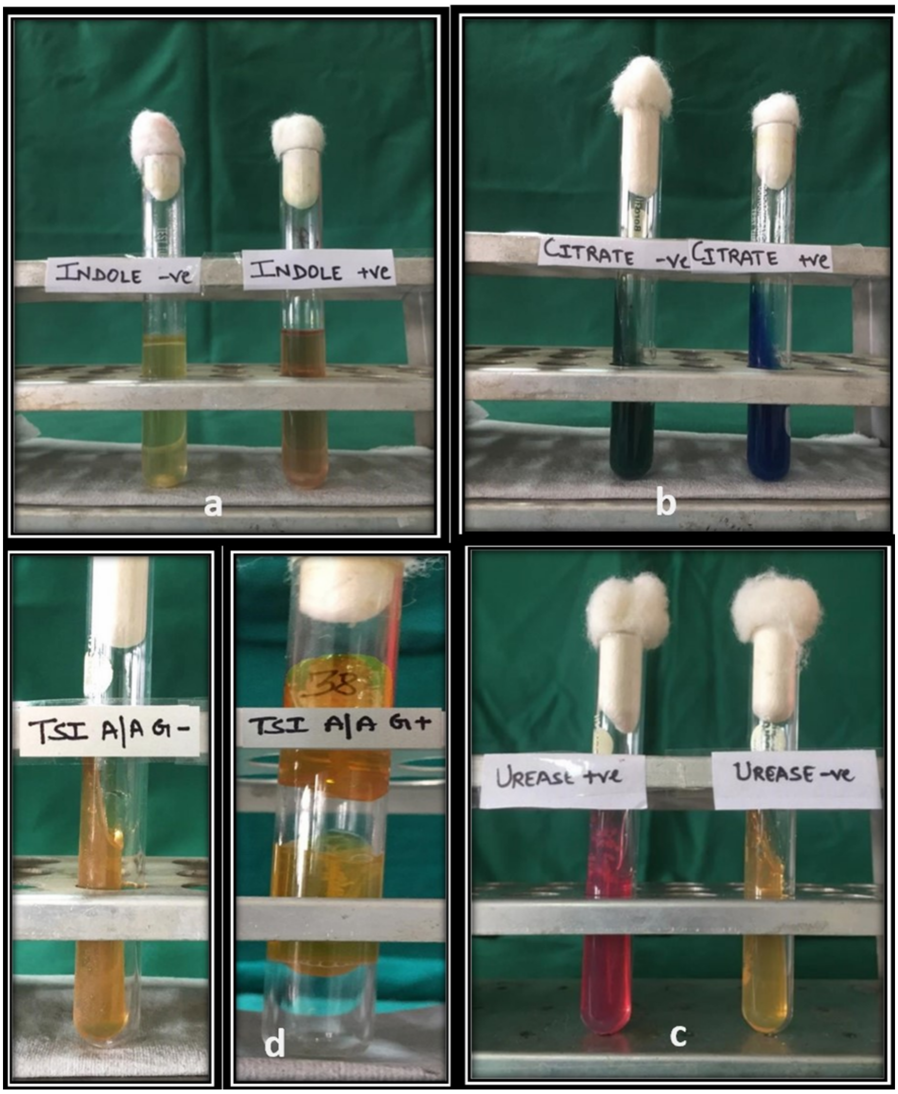

Figure 2 a-2d - Confirmatory biochemical tests - indole, citrate, urease, and TSI (Triple Sugar Iron) agar test

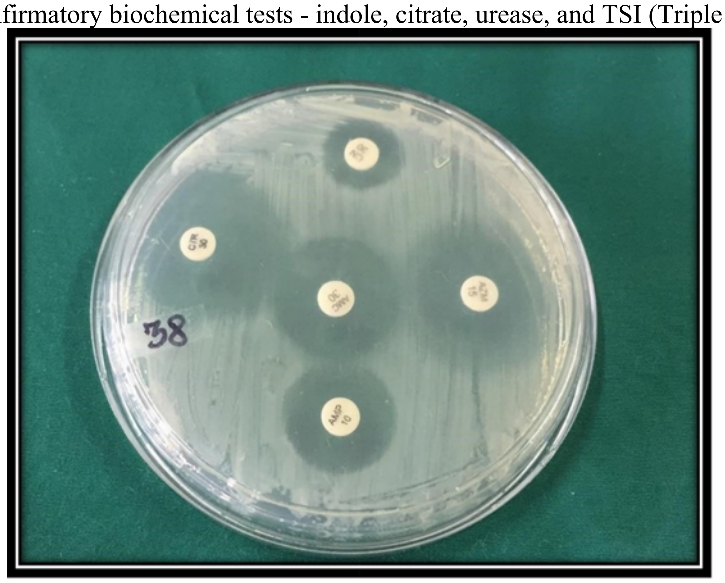

Figure 3 – Antimicrobial efficacy on Mueller Hinton agar medium through disk diffusion test

Figure 4 – Subject wearing a prefabricated orthodontic trainer appliance T4K-First Phase for habit interception

Figure Legends

Figure 1a-1b – Colonies obtained on the MacConkey agar plate; Photomicrograph revealing Gram staining of *Enterobacteriaceae* colonies
Figure 2 a-2d - Confirmatory biochemical tests - indole, citrate, urease, and TSI (Triple Sugar Iron) agar test
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Figure 4 – Subject wearing a prefabricated orthodontic trainer appliance T4K-First Phase for habit interception

Table legends

Table 1: Distribution of sample n (%)
Table 2: A comparison of the oral carriage of E. coli (n=52) and Klebsiella (n=12) pre- and post-habit intervention
Table 3: Antimicrobial susceptibility of E. coli and Klebsiella