Original Article

Microbial Distribution and Secretory IgA Level among Crossbite Patients at an Early Stage of Comprehensive Orthodontic Treatment

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Abstract

Background: Crossbite is any discrepancy in the relationship between the upper and lower teeth. Crossbites have a relationship with oral microorganisms, such as *S. mutans* and *lactobacillus* infections. Viral infections can spread to the oral cavity especially its soft tissue especially herpes simplex virus & cytomegalovirus. Secretory immunoglobulin represents a factor for acquired immunity in the oral cavity which is associated with oral infection and variation. **Objectives:** Isolation of microbes from crossbite patients, identifying by molecular techniques, determining sIgA level, and selecting appropriate antibiotics for the most common microbe. **Materials and Methods:** A total of 60 (30 samples of saliva and swab before Orthodontic Treatment and 30 samples of saliva after Orthodontic) during the period from October 2021 to April 2022, Sample were taken from patients and microbiological identification by microscopical and biochemical tests. An antibiotic sensitivity test for *S. mutans*. Genetic identification was detected in the *S. mutans* samples by using specificity *Sm479* primers, and *HCMV* primers for cytomegalovirus. Secretory IgA was determined by ELISA Kit. **Results:** A number of gram-positive bacteria were more than gram-negative (51% and 49%) respectively. The *S. mutans* and *Lactobacillus* are the most frequent among the other species, followed by *Candida*, *Klebsiella*, and *S. epidermidis*. 53% in male, while 47% in female, and age group (1315) was the most prevalent among samples 53%. Regarding *S. mutans*, the highest sensitive rate is Erythromycin 81%, followed by Amoxiclav 57%, Vancomycin 52%, and Nalidixic acid 43%. **Conclusion:** Most infectious cases were in ages (14-15) years. *S. mutans* is more prevalent among bacterial infections, molecular detection by PCR more specifically for *S.mutans* and CMV virus, sIgA level non-significant inverse correlations between age and sIgA level before treatment, while significant inverse correlation between age and sIgA level after treatment, and *S. mutans,* reported the highest sensitive rate is Erythromycin (81%), followed by Amoxiclav, Vancomycin,and Nalidixic acid.

Keywords: Antibiotics susceptibility, crossbite, *HCMV*, *Sm479*

Introduction

Crossbite is any discrepancy in the buccolingual relationship between upper and lower teeth.[1] The prevalence of crossbite has been reported to range from 7% to 22% in Western countries.3–6 In Nigeria, the prevalence has been reported to range from less than 2% to 16% in young children and adolescents.7–10 Crossbite has also been reported to occur more frequently in females.[2]

There are two types of crossbites; Posterior crossbite and anterior crossbite. Posterior crossbite if observed in mixed dentition, most likely will be observed in permanent dentition as well. It can have negative effects on the long-term growth of the maxillary and mandibular jaw.[3] While an abnormally reversed relationship between a tooth or teeth and an anterior crossbite is when the opposing teeth are positioned in the buccolingual or labiolingual orientation. The incidence of anterior crossbite varies between 4.5% and 9.5%, according to the numerous study groups.[4]

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Oral microbiology

The oral cavity contains some of the most varied and vast flora in the entire human body and it is the main entrance for two systems vital, the gastrointestinal and respiratory systems. Some of this normal flora is harmless, however, some of them can result in oral infections including tooth decay or gum disease. Bacteria, viruses, or fungi can all cause infections. A patient may get bacterial infections if they are exhausted or in a vulnerable condition, such as when they have diabetes, are taking steroids, have a compromised immune system, or have been taking antibiotics for an extended period of time. Long-term antibiotic use can also result in fungal diseases such candidiasis (oral thrush).[5]

Oral streptococci constitute the most oral bacterial groups in the human oral cavity.[6] *S. mutans* produces lactic acid and grows at low pH. As well as other microorganisms especially acid-producing bacterial species, Lactobacillus and Propionibacterium.[7] *S. mutans* is considered as a major pathogen of dental caries due to its ability to adhere and accumulate to the tooth surface.[8]

Candida is a part of the normal oral microbiota, being found in 17–75% human population, it can often be the cause of oral mycoses, especially in immunodeficient patients.[9] Some studies have compared *Candida* prevalence and *Candida* growth in orthodontic patients before, during, and after treatment.[10] *Candida albicans* is the most common opportunistic pathogens infecting patients. Patients with compromised host defenses are susceptible to which healthy people are exposed but usually resistant. Opportunistic conditions lead to oral mycosis, a fungal infection of the mouth. Impairment of host resistance enables local colonization in the oral cavity, which initiates and advances pathogenic conditions. With the rising use of immunosuppressive medications and immunodeficiency virus infections, oral mycosis has markedly grown in prevalence around the globe.[11]

The oral cavity is particularly susceptible to viral infections because of its conformation, particularly its soft tissue and salivary glands. Several viruses, including herpes simplex virus (HSV) and human papillomavirus (HPV), are associated with oral disease-causing primary lesions. Usually, the presence of viruses in the oral microbiota is indicative of potential infections, as the viruses are microscopic agents responsible for infection and damage to eukaryotic cells, with lifelong effects and/or recurrences.[12] Among the viruses commonly found at the oral sites, Epstein–Barr Virus (EBV) and Human Cytomegalovirus (CMV), as well as Herpes Simplex Virus (HSV), also showed significantly higher in patients with severe periodontitis than in healthy subjects.[13]

Secretory IgA

In fact, saliva's role in preventing dental cavities is one of its primary contributions. The pH of saliva varies from 6.2 to 7.6, is kept close to neutral, and is 6.7 on average. Proteins and peptides found in human saliva are necessary for the protection and maintenance of oral healthcare dynamics.[14] Saliva serves as a diagnostic tool for a variety of illnesses, due to the intracellular and extracellular pathways of saliva release in the oral cavity, such as systemic disorders, oral lichen planus, periodontal disease, dental caries, oral cancer, and more.[15]

Secretory IgA is a secretory factor for acquired immunity in the oral cavity. Antibodies of this type play a part in the maintenance of integrity. One of the most important immunoglobulins for defending against pathogens that invade mucosal surfaces is sIgA, which boosts oral immunity by inhibiting bacterial colonization and microbial adhesion.[16] Numerous studies have similarly shown that a low frequency of dental caries is correlated with high levels of sIgA.[17]

Recognition of sIgA antibodies in saliva may aid to identify liable patients before the development of orthodontically induced root resorption. The tipping movement is the easiest type of orthodontic tooth movement (OTM) which is formed when a single force is employed toward the crown of the tooth, for the duration of this movement the PDL are compressed (bone resorption) adjacent to the root apex on-the similar side of the applied force and the crest of alveolar bone on-the opposing side.[17]

Aim of study

study microbial distribution among crossbite patients at the early stage of comprehensive orthodontic treatment by the following:

1 -Isolation of microbes including bacteria and yeast from crossbite patients from the area of maxillary expansion at the early stage of comprehensive orthodontic crossbite
2 -Cross bite cases investigate if CMV infection correlates with the detection of microbes from the oral cavity.
3 -Identification of microbes using biochemical tests and molecular techniques.
4 -Selection of appropriate antibiotics for most common microbes on microbes to investigate which type of microorganisms have a high risk to the respiratory system to determine which type of antiseptic (mouthwash) must be used indicate during the period of treatment
5 -Detection the role of Secretory IgA.

Materials and Methods

Sample collection

Total of 60 (30 samples of saliva and swab before Orthodontic Treatment and 30 samples of saliva after Orthodontic. These cases were collected from the Orthodontic department - college of dentistry –University of Babylon. The samples were collected from both sexes,

Table 1: Specific primers sequence and amplicon size

Primer Name	Sequence	Product size(bp)	Reference
Sm479	F: TCGCGAAAAAGATAAACAAACA R: GCCCCTTCACAGTTGGTTAG	479	Zhou *et al.*, 2007
HCMV	F 5'-ACGTGTTACTGGCGGAG TCG-3' R 5'-TTGAGTGTGGCCAGACTGAG -3'	368	Blankson *et al.*, 2018

Table 2: Distribution of infections according to gander

Age group	No of patients total		
	Male (%)	Female (%)	Total
7 9	3 (10%)	1 (3%)	4 (13%)
10 12	6 (20%)	4 (13%)	10 (33%)
13 15	7 (23%)	9 (30%)	16 (53%)
Total	16 (53%)	14 (47%)	30 (100%)

during the period from October 2021 to March 2022. The age group is from (7-15) years old.

Microbiological Identification

microscopical and biochemical tests were done for all samples by using certain culture media such as blood agar and MSA agar.

Antibiotics Sensitivity Test

for *S. mutans* isolates by utilizing the Kirby-Pour technique, a pure culture of previously identified isolated microorganisms performed a disc diffusion test. The inoculum on the Mueller Hinton agar plate contaminated the use of a sterile swab to the inoculum from the bacterial suspension. Using flammable forceps, the antibiotic discs were positioned on the medium's surface and incubated for 24 hours at 37°C. Using a ruler, areas of inhibition were measured and the areas of inhibition were compared. (CLSI, 2020). Erythromycin (30), Amoxiclav (10), Vancomycin (30), and Nalidixic acid (30) were tested for *S. mutans*.

The immunological study

using Secretory IgA ELISA Kit. This ELISA kit uses the Sandwich-ELISA principle. All samples (before and after treatment) were tested for single and mix infections.

Molecular Identification of *S.* mutans

From bacterial cultures on Mitis Salivary Agar (MSA) cultivated in Brain Heart Infusion broth (BHI), DNA was extracted in accordance with manufacturer-recommended procedures using PCR and the species-specific primer Sm479. The reaction was conducted as follows: 94 °C for 3 min, then 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 59 s, followed by 5 min at 72 °C as an extension. as shown in [Table 1]

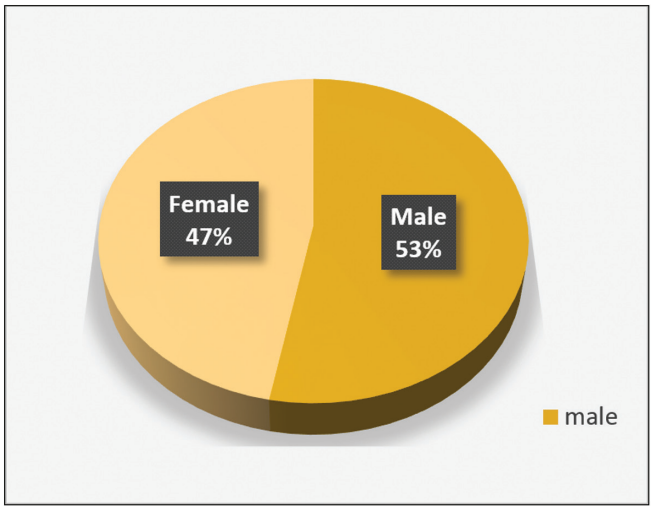

Figure 1: Distribution of infections according to gander

Of cytomegalovirus

DNA was extracted from all patients swaps according to protocols recommended by the manufacturer; PCR technique done using species-specific primer *HCMV*.as shown in [Table 1]

Ethical approval

The study was carried out in accordance with the ethical standards that have their origin in the Helsinki Declaration. Before a sample was taken, it was done with the patient's verbal and analytical consent. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 566 (in 3/1/2022) to get this approval.

Results

According to the results, the sample taken from patients with a crossbite, the subjects were divided into two groups male and female, 16 (53%) of all these groups were male, while 14 (47%) in female [Figure 1, Table 2].

Identification of microorganisms

As a result of this study, 36 (51%) of all samples (after or before treatment) were Gram-positive bacteria, while 34 (49%) were Gram-negative bacteria [Figure 2].

[Table 3] noted the microbial distribution after and before the treatment, the *S. mutans*, and *Lactobacillus* are the most frequent among the other species.

Table 3: Distribution of infections after and before the infection

	Mixed infection		Single infection	
	Microorganisms	**No.**	**Microorganisms**	**No.**
Before	**Strep+*Staph*	4(13%)	*Lactobacillus*	5(17%)
	**Lactobacillus+Strep*	3(10%)		
	Lactobacillus+ Staph	1(3%)	*S.mutans*	4(13%)
	Lactobacillus+ Strep +Staph	1(3%)		
	*Strep+*Klebsiella*	3(13%)	*Klebsiella*	2(7%)
	Staph+ Klebsiella	1(3%)		
	Lactobacillus+ Klebsiella	1(3%)	*Staph.aureus*	2(7%)
	*Klebsiella+*Candida*	1(3%)		
	Lactobacillus +Candida	1(3%)	*Staph.epidermidis*	1(3%)
Total		16 (53%)		14 (47%)
After	**Strep.mutans*	0 (0%)	*Lactobacillus*	7(23%)
	Strep.mutans+Lactobacillus	1(3%)	*S. mutans*	6(20%)
	Lactobacillus+ Candida	1(3%)	*Klebsiella*	4(13%)
	**Staph aureus*	1(3%)	*Staph.epidermidis*	3(10%)
	Klebsiella	2(7%)	*Staph.aureus*	3(10%)
	Klebsiella+ Lactobacillus	1(3%)		
	Klebsiella +fungi+*CMV	1(3%)		
Total		7 (23%)		23 (77%)

The bacteria specimens were cultured on MSA agar and blood agar [Figure 3]. *S. mutans* appeared as alpha- or gamma-hemolytic on blood agar plates, also on MSA agar was similar to the blood agar results. Under the microscope, *S. mutans* appears as a chain of cocci bacteria and is gram-positive. *Candida albicans* were examined under a microscope, budding of *Candida albicans*, and Germ tube formation [Figure 4], as well as the colonies, gave positive results on the catalase test.

Measurement of the IgA level

In this study, levels of the IgA in males were (399.5399) before treatment and was (309.5284) after treatment, while in females was (308.6109) before treatment and was (275.9641) after treatment. [Table 4] shows, the levels of the IgA in a single infection were (281.8252) before treatment and was (292.8528) after treatment, while in mixed infection was (443.1420) before treatment and was (330.5215) after treatment.

Antibiotics susceptibility

In this study, *S. mutans,* reported the highest sensitive rate is Erythromycin (81%), followed by Amoxiclav (57%), Vancomycin (52%), and Nalidixic acid (43%) [Figure 5]

Genetic detection

In this study, twenty-three of *S. mutans* samples which identified by biochemical tests applied with *SM479*. Twenty-one isolates gave positive results for this gene, and 2 isolates failed to form bands via agarose gel electrophoresis [Figures 6 and 7].

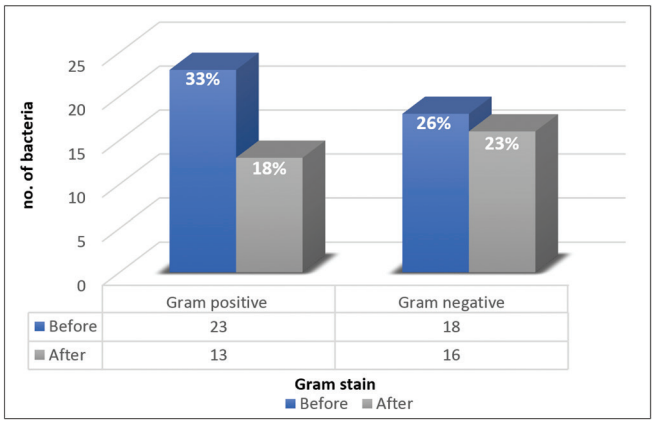

Figure 2: Distribution of bacteria according to Gram stain

All study samples were tested for the *HCMV* gene, seven of these samples gave positive results for this gene.

Discussion

Lemmen *et al*., (2004) demonstrated that gram positive bacteria cause oral infections more than gram-negative bacteria.[18] Sometimes some cases of crossbites are synergic with missing molars, whereas the Asymmetric molar relation could contribute to a crossbite.[19] Adults have a high risk of developing gingivitis, especially an increase in the number of Streptococci and Lactobacilli.[20] *S. mutans* are commonly found in the human oral cavity and are a major contributor to tooth decay. The result of decay can greatly affect the overall health of the individual. These facultative anaerobes gram stains which appear as gram-positive short or long chains

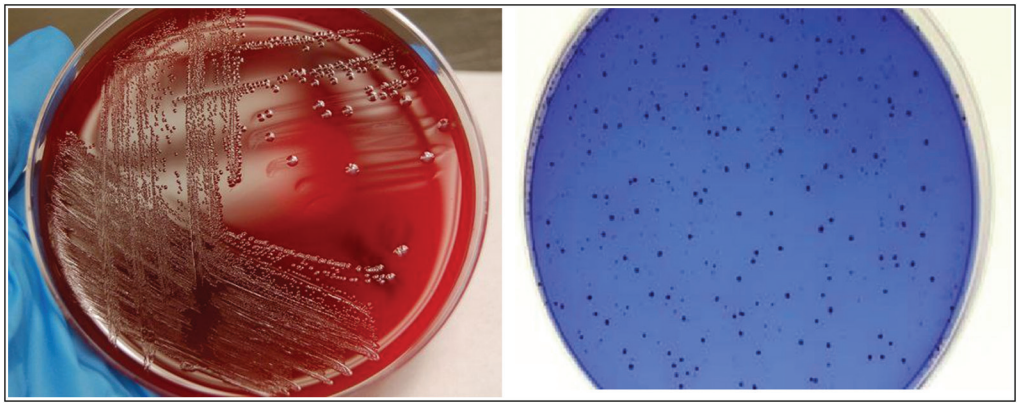

Figure 3: *S. mutans* on blood agar and MS

Table 4: Measurement of the IgA level according to the gander and type of infection

		Measurement of the IgA level		
		Before	After	p
Gander	**Male**	399.5399 ± 44.46288	309.5284 ± 25.88042	0.090
	Female	308.6109 ± 38.26331	275.9641 ± 21.92095	0.466
	P	0.138	0.338	
Type of infection	**Single**	281.8252 ± 37.46285	292.8528 ± 42.94475	0.868
	Mixed	443.1420 ± 38.62524	330.5215 ± 19.36581	0.218
	P	0.006	0.531	

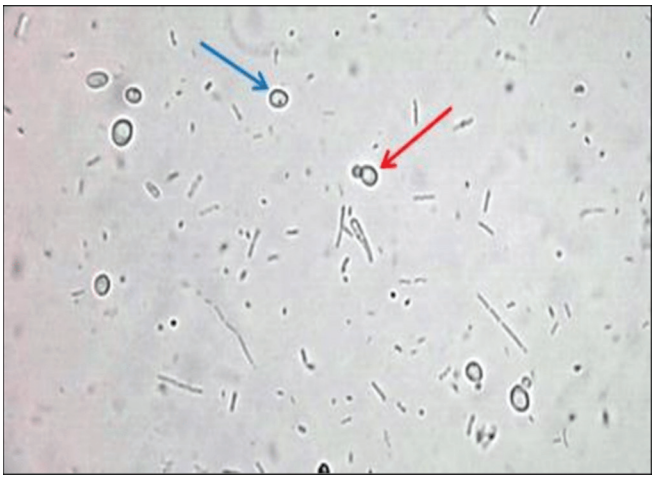

Figure 4: *Candida albicans* under microscope (40X)

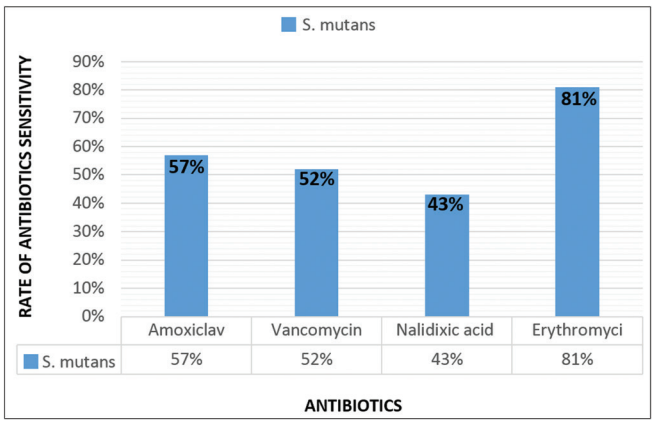

Figure 5: Sensitivity of *S. mutans* isolates to antibiotics

with a rough or frosted-glass appearance *S. mutans* appearance was rough in MSA agar media and usually have bead.[21,22] *Candida albicans* appear as clustered cells under the microscope as well as filamentous morphology embedded in a flocculent extracellular material, called a germ tube and positive catalase.[23] An increase in *Staph. epidermidis*, *Klebsiella*, and *Lactobacillus* significantly increased after three months of treatment, that is because the insertion of the fixed appliance may considerably alter the oral microenvironment since it provides bacterial colonization and accumulation areas for dental biofilm and fungi.[24,25]

The frequency distribution of gender of the patients with a dental crossbite, which is evident that the male population has a high prevalence of crossbite (60.06%).[26] some studies found non-significant among males and females school children in Brazil, among the 13 to 17 age group.[27]

The IgA levels

The IgA level increase after treatment in a single infection, while in mixed infection the IgA level decrease after treatment, this is due to the secretory immunoglobulin A (sIgA) is a key antibody in the salivary defense system, it may respond to changes in the oral microenvironment during orthodontic treatment.[27] The sIgA level in the case group before and after treatment according to the gander.

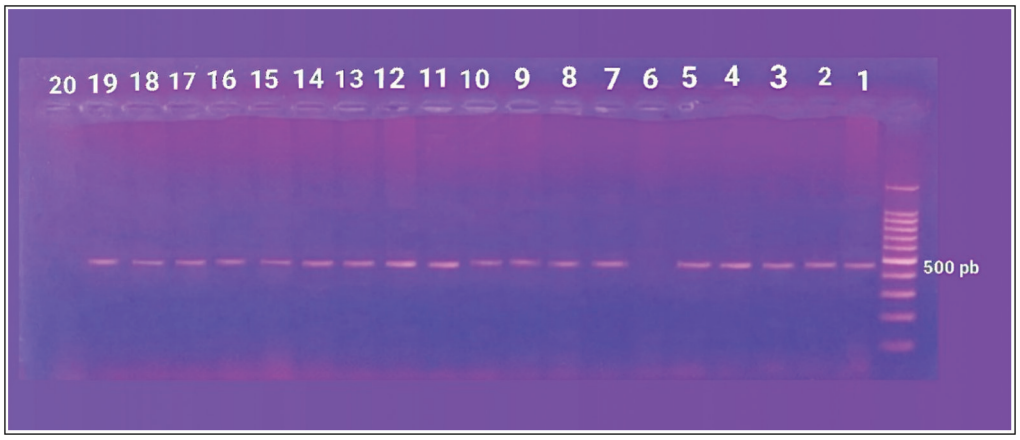

Figure 6: Gel electrophoresis of PCR product of specificity *Sm479* primers by PCR. DNA amplification was observed from the *S. mutans* isolates numbered (1-19) were positive

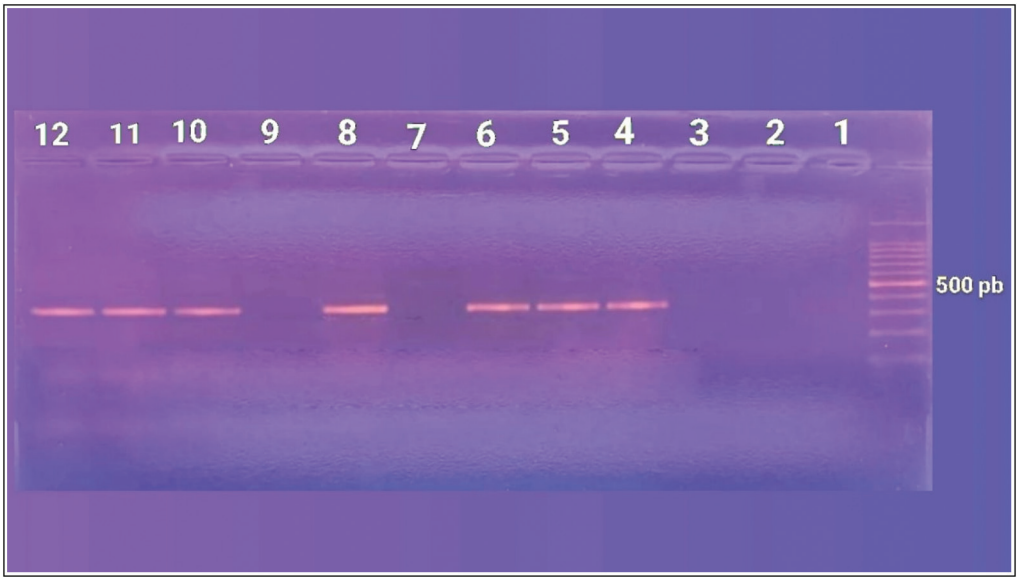

Figure 7: Gel electrophoresis of PCR product of specificity *HCMV* primers by PCR. DNA amplification was observed numbered (4, 5, 6, 8, 10, 11, 12) were positive

There is no high significance between male and female patients that due to the activation or increased IgA level depending on the microbial infections (bacterial or viral) in the mouth.[26]

In this study, we found there are non-significant inverse correlations between age and IgA level before treatment, while significant inverse correlation (p 0.027) between age and IgA level after treatment. This difference in inverse correlation is significant due to the different values of bacterial content before and after treatment. Saliva is considered to contain innate immune factors and various salivary defense proteins. Since secretory immunoglobulin A (sIgA) is a key antibody in the salivary defense system, it may respond to changes in the oral microenvironment during orthodontic treatment, with no relationship with gender.[28]

Antibiotics susceptibility

Streptococcus mutans

According to many of study, (45%) of *S. mutans* are sensitive to vancomycin, *S. mutans*-associated tooth decay and vancomycin resistance are one of the most prevalent and costly infectious diseases in the United States.[29] Amoxiclav sensitive in this study more than some studies, that's maybe because most species of the *S. mutans* in their study confirmed the presence of biofilm in addition to the difference in the age group range.[30] *S. mutans* strain is considered one of the most important and predominant pioneer species in the oral cavity and demonstrated resistance to erythromycin (25%). The elevated rate of erythromycin resistance in children isolates can be explained by selective antibiotic pressure, in which erythromycin is used more common in children than in adults, and this may play an important role in

the emergence and spread of erythromycin resistance in children isolated.[31] Nalidixic acid in this study recorded the less sensitive percentage, 43% of the resistance of isolates to nalidixic acid increased among isolates (32%) because of biofilm formation.[31]

Genetic identification

SM479 gene which was used for the identification of *S. mutans* from all bacterial species found in the environment of the oral cavity. According to this study, 35% of all samples (before and after treatment) gave positive results for the SM479 gene via gel electrophoresis. Aljarah *et al*. (2018) found (that 51.5%) of samples were positive for S. mutans (positive SM479 gene).[32]

The majority of people contract CMV in childhood, but it rarely results in illness. Usually, 30% to 40% of children aged 1 to 3 years in childcare settings excrete CMV (sometimes can be up to70%).[33,34] By coming into close touch with biological fluids like blood, urine, or saliva, CMV can be transferred from one person to another. CMV can also be transmitted from a mother to her unborn child. The risk of infection among children and staff in educational facilities is very high. CMV infection can spread for months to years in some people; it can for a relatively short period of time in others.[34]

Cytomegalovirus-seroprevalence increased with age (21.5% at ages 1–2; 32.0% at ages 14–17). Cytomegalovirus-seropositivity was significant associated with migration background, country of origin, and place of birth, and (among migrants only) with low socioeconomic status. Also, the transmission influences CMV-seroprevalence among preadolescent children, as well as breastfeeding increased the risk of CMV-seropositivity.[35-37]

Conclusion

The crossbite has a significant effect on the patient through the increase in the microbial content. The treatment especially in the early stage can reduce the risk of microbial infection and stimulate the sIgA as a line of defense. *Streptococcus mutans*, *Lactobacillus*, *Klebsiella*, and *Staph. epidermidis* is the most common bacteria that are isolated from crossbit patients whether before or after treatment, as well as Cytomegalovirus (CMV) isolated from Saliva. The most infectious cases were in ages (13-15) years.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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