Khalij Libya Journal of Dental and Medical Research. 2026;10(2):175-184
https://doi.org/10.47705/kjdmr.26215
eISSN:2708-888X

Original article

Effectiveness of 0.06% Chlorhexidine-based Mouthwash against Certain Oral Microflora among Patients Undergoing Fixed and Removable Orthodontic Treatment

Zakaria Khmaj[1]*, Mofida Khmaj[2], Abdulfatah Khmaj[2], Rawan Albeshti[3]

[1]*Belarusian State Medical University, Minsk, Republic of Belarus.*
[2]*Faculty of Dentistry, Zawia University, Alzawiyah, Libya.*
[3]*Libyan Authority for Scientific Research, Tripoli, Libya.*
***Corresponding Email**: Zakaria833@gmail.com*

ABSTRACT
This study was conducted to evaluate the effectiveness of 0.06% chlorhexidine-based mouthwash available in local pharmacies when they were prescribed for use by patients undergoing orthodontic treatment. Fifteen patients undergoing fixed or removable orthodontic treatment were selected. Swabs were taken from five patients with ligature brackets, five patients with self-ligating, and the other five patients with removable orthodontic appliances. The concentration of the chlorhexidine-based products was 0.06%, and the culture method, as a fundamental microbiological technique, was chosen, which is based on the cultivation of microorganisms on specific nutrient media. The morphological and tinctorial analyses were also used to study the structure and shape of cells or tissues, as well as their staining with special dyes for subsequent microscopic examination. Further, the disk diffusion method (Kirby-Bauer Method) is based on measuring the zone of inhibition of microbial growth around disks impregnated with antibacterial agents placed on a nutrient medium. The collected data were analysed using Microsoft Excel and Statistica 10.0 software. Descriptive statistics were used to summarise the obtained results for each group. Oral microorganisms were identified in patients treated with conventional ligature brackets, self-ligating brackets, and removable orthodontic appliances. Differences in the composition and distribution of oral microorganisms were observed among the three studied groups, including variations in the prevalence of Gram-positive/negative cocci, Gram-positive/negative rods, spore-forming bacteria, and Candida species. 0.06% chlorhexidine-based mouthwash demonstrated high antibacterial activity when compared to chlorhexidine-free mouthwash; thus, it proved to be more effective in reducing the oral microflora growth. The mean inhibition zone diameters were 20.80 mm, 16.57 mm, and 19.67 mm in Groups 1, 2, and 3, respectively. It was concluded that the correct selection of antibacterial products and patient motivation to maintain oral hygiene play a critical role in preventing the accumulation of pathogenic microflora, particularly in patients undergoing orthodontic treatment, where the biofilm accumulation is significantly increased.
Keywords. *Chlorohexidine-based mouthwashes, Orthodontic Treatment, Oral Microflora, Resistance, Sensitivity.*

***Received**: 25/04/26*
***Accepted**: 22/06/26*
***Published**: 30/06/26*

Introduction

Orthodontics is an essential field for modern dental treatment [1]. Fixed or removable orthodontic treatment remains the treatment of choice for many patients; however, this treatment promotes the accumulation of bacteria, plaque, and food debris around the orthodontic appliance and also on the surface of the removable orthodontic device, leading to unfavourable outcomes [2-3]. Further, the proper selection and evaluation of the effectiveness of dental hygiene products intended for controlling oral microflora is becoming increasingly relevant. These products must meet specific requirements, such as efficacy against pathogenic microorganisms, the ability to minimise the risk of complications such as gingival inflammation, enamel demineralisation, increased plaque accumulation, and orthodontic treatment-associated oral infections [1-2-4].

Orthodontic treatment has a crucial role for correcting abnormalities in tooth and jaw positioning, thereby enhancing the functional efficiency of the dentoalveolar system and achieving a harmonious aesthetic outcome; including: (1) correction of malocclusion and management of occlusal discrepancies; such as cross-bite, open-bite, and deep-bite to ensure proper intercuspation and improved masticatory function; (2) improvement of aesthetics by achieving a balanced and symmetrical dental alignment, enhancing smile appearance and overall facial aesthetics; (3) improvement of oral hygiene with optimised tooth positioning facilitates access for daily hygiene procedures, reducing the risk of caries, periodontitis, and other oral diseases; and (4) prevention/management of functional disorders, which addresses the complications associated with malocclusion, as speech disturbances and temporomandibular joint (TMJ) dysfunction as seen in (Figure 1)[5-6].

Khalij Libya Journal of Dental and Medical Research. 2026;10(2):175-184
https://doi.org/10.47705/kjdmr.26215
eISSN:2708-888X

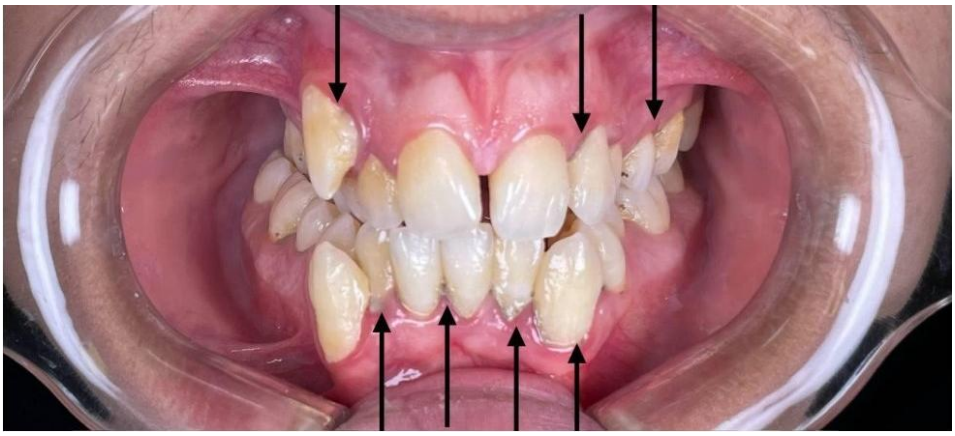

Figure 1. 20-year-old patient before orthodontic treatment: the initial examination revealed narrow maxillary and mandibular arches, crowding in both dental arches, and ectopic eruption of the canines. All these factors contributed to dental plaque formation (as indicated by arrows).

Both orthodontic appliances, fixed and removable, promote food impaction and create conditions for plaque accumulation, which complicates oral hygiene maintenance and increases the risk of developing conditions such as gingivitis, periodontitis, dental caries, and white spot lesions [7]. A previous study conducted by Simona Santonocito and Alessandro Polizzi found that three months after the initiation of orthodontic treatment, the number of certain periodontopathic bacteria, such as *P. gingivalis*, significantly increased, compared to the control group not undergoing orthodontic treatment [7]. Of particular interest is the study by Naranjo, which evaluated the relationship between changes in clinical parameters and subgingival plaque using culture analysis of subgingival microbial samples collected from patients before and after bracket bonding, as well as from the control group without braces. It was found that three months after starting the orthodontic treatment, the levels of *P. gingivalis*, *P. intermedia/Prevotella nigrescens*, *T. forsythia*, and *Fusobacterium* species were higher compared to both the control group and baseline values [6-7].

Alongside, various clinical studies have also reported an increase in the presence of *S. mutans* and *Lactobacillus* species, which are responsible for the development of dental caries and white spot lesions on the tooth surfaces during orthodontic treatment, as shown in (Figure 2) [6-8]. Therefore, the main aim of the current study was to evaluate the efficacy of 0.06% chlorhexidine-based mouthwash on oral microorganisms for patients undergoing fixed and removable orthodontic treatment.

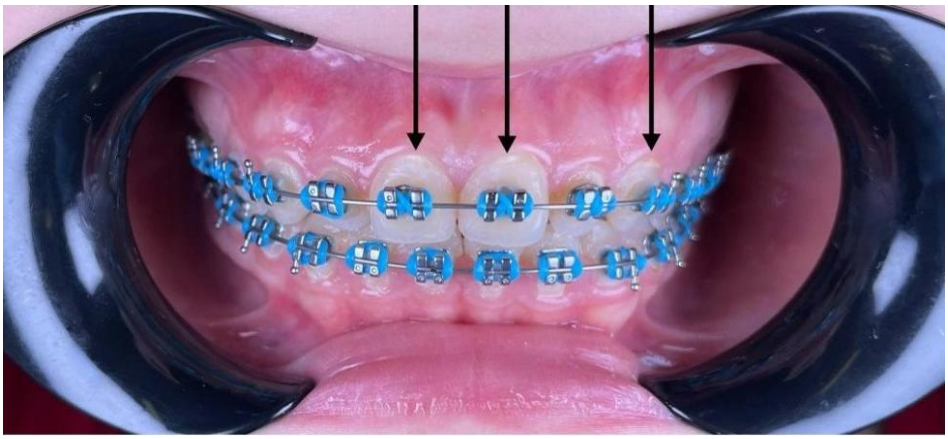

Figure 2. Development of white spot lesions on the tooth surfaces during orthodontic treatment.

Materials and methods

Study Design

The study included fifteen patients, who were divided into three groups; the first group received treatment using a ligature edgewise bracket system, the second group used a self-ligating bracket system, and the third group consisted of patients undergoing removable orthodontic treatment.

Inclusion Criteria

The general inclusion criteria were as follows:

I. Male and female participants aged 11–35 years.

Khalij Libya Journal of Dental and Medical Research. 2026;10(2):175-184
https://doi.org/10.47705/kjdmr.26215
eISSN:2708-888X

II. Good general health status confirmed by medical history.
III. Provision of written informed consent to participate in the study.

Additional inclusion criteria included:

IV. Oral rehabilitation before appliance placement, comprising professional oral hygiene procedures and treatment of existing dental and periodontal conditions.
V. No active gingival inflammation prior to appliance placement.
VI. Minimum of 6 months of ongoing orthodontic treatment.
VII. Adherence to orthodontist's daily preventive instructions, including brushing after each meal.

Exclusion Criteria

I. Periodontal therapy or antibiotic use within the previous 6 months.
II. Systemic diseases affecting periodontal health or treatment outcomes, such as diabetes mellitus, HIV infection, or cancer.
III. Conditions requiring antibiotic prophylaxis before periodontal procedures, such as certain cardiovascular diseases or joint replacement surgery.
IV. Chronic use (≥2 weeks) of medications affecting periodontal tissues, including phenytoin, calcium channel blockers, cyclosporine, nonsteroidal anti-inflammatory drugs (NSAIDs), and anticoagulants.
V. Ongoing anticoagulant therapy that cannot be safely discontinued (e.g., oral thrombin inhibitors).
VI. Pregnancy or lactation, due to hormonal effects on periodontal status.

Preparation of Culture Media for Microorganism Cultivation

The preparation of culture media involved dissolving and reconstituting their components. Dehydrated (powdered) media were prepared by dissolving the required amount of powder in flasks filled with distilled water. The mixture must be thoroughly stirred to ensure complete homogeneity. For media containing agar as a solidifying agent, reconstitution was performed under gentle heating with continuous stirring. During preparation, the temperature was maintained at 95-100°C; however, brief boiling (for less than 1 min) was permitted only to prevent burning of the medium. Prior to pouring, the medium was cooled to 45-50°C, as agar solidifies at temperatures below 32°C [9].

In this study, Meat-peptone agar (MPA) based on enzymatic beef hydrolysate was used for the cultivation of microorganisms. The MPA is widely used for the cultivation and characterisation of microbial growth. It enables the assessment of colony morphology, including size, shape, margins, colour, surface sheen, transparency, and consistency among other phenotypic characteristics [9].

Materials Collection for Bacteriological Examination

The oral cavity swabs were collected, and the samples were prepared for oral microbiota; also, a few instructions were followed:

I. No food intake before the analysis.
II. No fluids for 2 hrs before the analysis.
III. No teeth brushing or gargling before the sample collection.

For sample collection, wooden sticks with cotton tips were used in sterile 12×150 mm polypropylene tubes. The cotton tip was applied to the surface of the buccal mucosa and gingiva to collect sufficient biological material from the oral cavity for 30 secs. Then, each sample was transferred into a tube containing a liquid transport medium (2 mL sodium chloride solution). The collected biomaterial was transported to the laboratory in special thermal containers at a temperature not higher than –4°C within 12 hrs for further analysis.

Isolation of Microorganisms on Nutrient Media

For the identification of the obtained microorganisms, Gram staining was used. A small amount of the bacterial culture was transferred onto a defatted glass slide using a flamed and cooled inoculating loop and spread into a thin smear. The smear was then air-dried and, after complete drying, fixed by passing through a flame [9]. Staining was performed according to the following protocol:

I. Methylene blue was first applied to the fixed smear for a few minutes.
II. The stain was decanted, and the smear was immersed in Lugol's iodine solution for two minutes until the preparation darkened.
III. The smear was rinsed with alcohol solution by repeatedly adding and decanting it until the smear was decolorised and the liquid ran clear (it took approximately 60 secs).
IV. The slides were thoroughly washed with distilled water.

To detect Gram-negative bacteria in the smear, additional staining with fuchsin was performed. Further, the dye was applied for 2 mins. At the end of the procedure, the slide was rinsed and dried. All the Gram-

Khalij Libya Journal of Dental and Medical Research. 2026;10(2):175-184
https://doi.org/10.47705/kjdmr.26215
eISSN:2708-888X

positive microorganisms stain dark violet, while the Gram-negative microorganisms stain red. Spherical forms are usually Gram-positive, whereas spiral forms are typically Gram-negative.

Kirby-Bauer Disk Diffusion Method

The sensitivity of microorganisms to antibacterial agents was determined using the disk diffusion method (DDM), which was developed by William Kirby and Alfred Bauer. The principle of this method is based on the ability of the agent to diffuse from impregnated paper disks into the nutrient medium, inhibiting the growth of microorganisms seeded on the agar surface [10].

Mueller-Hinton Agar (MHA) was used as the standard medium for susceptibility testing using the DDM. Filter paper disks were used, each impregnated with 4.0 µL of dental mouthwash. To increase the concentration of the agent, the disks were dried and impregnated 15 times [10].

Next, 0.5 mL of a bacterial suspension with turbidity equivalent to 0.5 McFarland standards (approximately 1.5×10^8 CFU/mL) was inoculated onto the surface of the MHA in a Petri dish. The suspension was evenly spread using a spatula. Four disks containing 0.06% chlorhexidine-based dental hygiene solution and a chlorhexidine-free daily-use peppermint essential oil mouth wash were placed on the agar surface. The plates were then incubated at 37°C for 24 hrs [10]. After incubation, the results were evaluated by measuring the diameter of the zone of inhibition around each disk in millimeters.

Determination of Susceptibility of Isolated Microorganisms & Interpretation of Results

The zone of inhibition indicates the sensitivity of microorganisms to a specific antiseptic agent. When measuring inhibition zones, the zone of complete growth inhibition (Figure 3), visible to the naked eye at a viewing distance of approximately 30 cm, was used as a reference.

For measurement, the Petri dish with the lid closed was placed upside down on a dark matte surface so that light fell on it at a 45° angle [10]. The inhibition zones were measured in millimeters using a ruler with 1 mm accuracy.

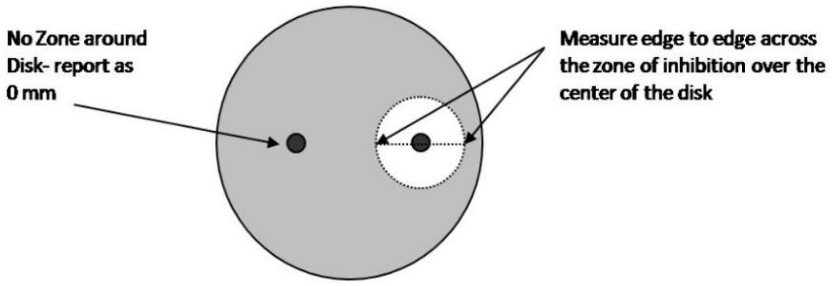

Figure 3. Determination of microorganisms' sensitivity using DDM [10].

Measurement of inhibition zone diameters was performed to the nearest millimeter and based on approximately 80% growth inhibition. Microcolonies located at the border or within the inhibition zone, as well as insignificant growth inhibition (< 20%), were not considered. The results were evaluated according to the criteria as presented in (Table 1), which contains the breakpoint values of inhibition zone diameters for resistant, intermediate, and susceptible strains.

The obtained data were entered into Microsoft Excel and Statistica 10.0 software for processing and analysis. Descriptive statistical methods were applied to organise and summarise the results. Inhibition zone diameters were recorded in millimeters and tabulated for each microbial isolate. Mean values, ranges, and frequencies were calculated where appropriate. Based on the established breakpoint criteria, the isolates were classified as susceptible, intermediate, or resistant to the tested antimicrobial agents. The resulting data were then compared descriptively among the studied groups to evaluate the differences in antimicrobial susceptibility patterns and microbial composition.

Table 1. Criteria for results interpretation to determining microbial susceptibility to antibacterial oral hygiene agents.

	R ≤ 14	**I = 15-16**	**S ≥ 17**	**0**
Growth Inhibition Zone Diameter/mm	Resistance	Intermediate	Susceptible	Absence of growth

Khalij Libya Journal of Dental and Medical Research. 2026;10(2):175-184
https://doi.org/10.47705/kjdmr.26215
eISSN:2708-888X

Results

Oral swab samples were inoculated onto MPA nutrient medium. A total of 15 Petri dishes (3 groups) were used. The growth of 1-4 types of microbial colonies was observed on the plates. To obtain pure cultures, microorganisms were sub-cultured onto fresh MPA plates for further incubation. Using Petri dish No. 3 as an example of microbial growth and sub-culturing, the procedures performed for all cultured samples are presented in (Figure 4).

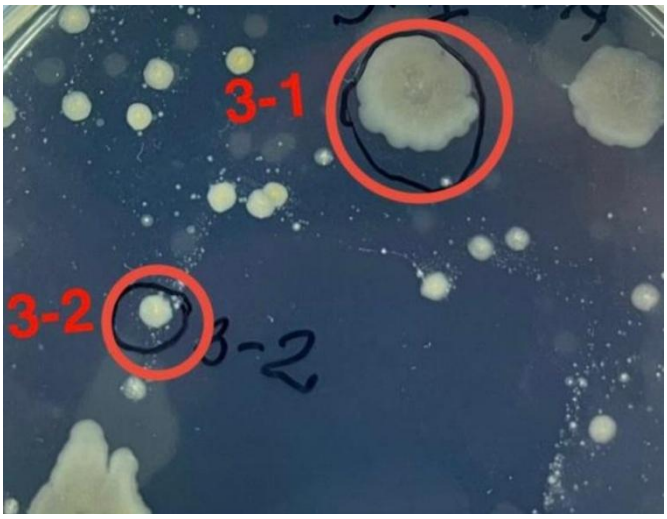

Figure 4. Microbial growth in meat-peptone agar.

For the identification of microorganisms from the obtained colonies, smears were prepared and examined under a microscope using oil immersion. The results of microscopic examination of smears are seen in (Figure 5a. & b).

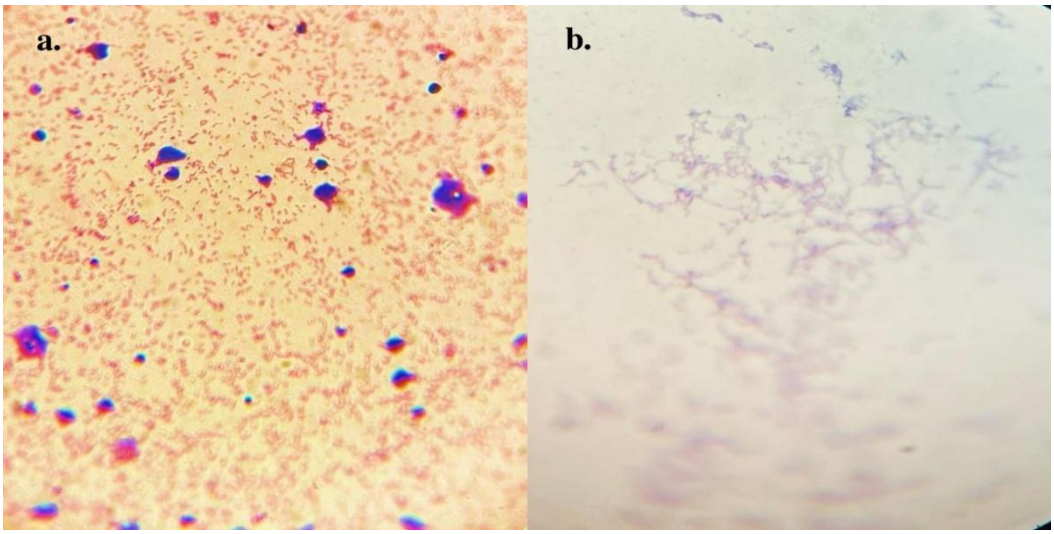

Figure 5a. Smear 3.1 – Gram-negative cocci; (b.) Smear 3.2 – Gram-positive rods.

Overall, Gram-positive cocci were the most frequently detected microorganisms, with counts of 10 in Group 1, 9 in Group 2, and 9 in Group 3 (total n = 28). This indicates dominating the oral microbiota across all groups and likely represents a stable core flora. Other bacterial groups were observed at much lower frequencies, as tabulated in (Table 2). Gram-positive rods were detected mainly in Group 2 (n = 3), with fewer in Group 1 (n = 2) and none in Group 3 (total n = 5). Gram-negative rods showed a different distribution, being absent in Group 1, present in low numbers in Group 2 (n = 1), and more frequent in Group 3 (n = 3). Gram-positive spore-forming rods were rare and found only in Groups 1 and 2 (n = 1 each, total n = 2), while Gram-negative spore-forming rods were detected only once in Group 2. Candida species were also infrequently identified, appearing only in Groups 1 and 2 (n = 1 each, total n = 2) and being absent in Group 3. Notably, Group 2 demonstrated the highest microbial diversity, as it contained all identified microorganism types, including Gram-positive and Gram-negative bacteria as well as spore-forming forms and Candida, which suggests a broader spectrum of oral microbial colonisation compared with other groups.

Khalij Libya Journal of Dental and Medical Research. 2026;10(2):175-184
https://doi.org/10.47705/kjdmr.26215
eISSN:2708-888X

Table 2. Microbiological Identification Results of Oral Swab Samples.

Microorganisms	1st Group	2nd Group	3rd Group	Total
Gram-positive cocci	10	9	9	28
Gram-negative cocci	1	1	1	3
Gram-positive rods	2	3	0	5
Gram-negative rods	0	1	3	4
Gram-positive spore-forming rods	1	1	0	2
Gram-negative spore-forming rods	0	1	0	1
Candida	1	1	0	2

The effectiveness of 0.06% chlorhexidine-based mouthwash and chlorhexidine-free mouthwash was evaluated using DDM, as seen in (Figure 6). Using this method, all microorganisms isolated from the 15 patients were examined as mentioned in the materials and methods section.

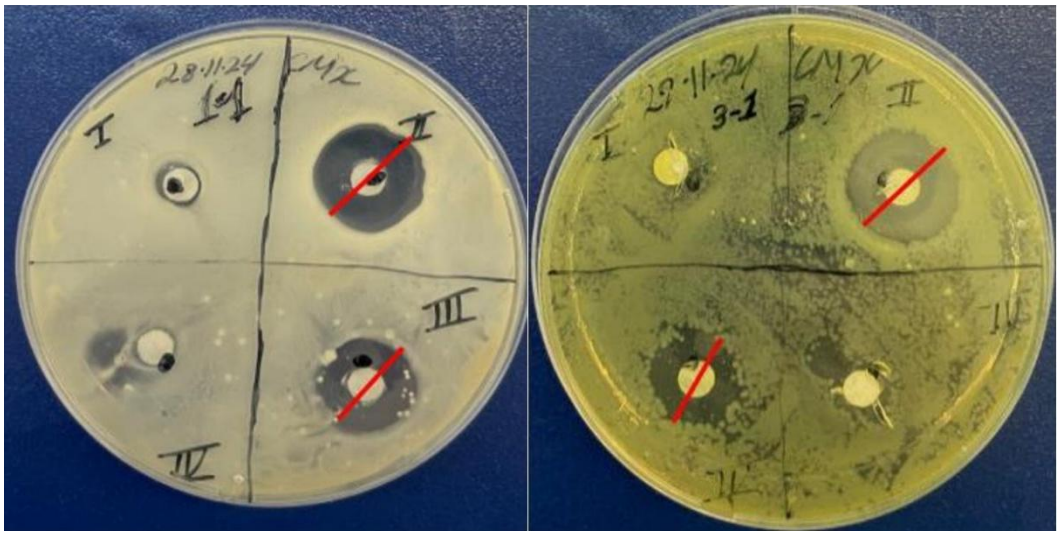

Figure 6. Efficacy of antibacterial oral hygiene products using DDM.

Based on the analysis of the data presented in Table 3 of the first experimental group, the following conclusions can be made: 0.06% chlorhexidine-based mouthwash showed significant inhibition of the growth of most microorganisms, including Gram-negative and Gram-positive cocci, rods, as well as Gram-positive spore-forming rods and Candida species. Further, the average diameter of the inhibition zone was 20.80 mm.

Table 3. Bactericidal activity of 0.06% chlorhexidine-based mouthwash and essential oil-based mouthwash against identified microorganisms of the 1st group.

1st Experimental Group		0.06% Chlorhexidine-based mouthwash		Essential oil-based mouthwash	
		Diameter of the growth inhibition zone, mm			
Sample No.	Microorganisms	R ≤ 14, I = 15-16, S ≥ 17			
1.2	Gram-positive cocci	23	S	18	S
1.4	Candida	20	S	14	R
3.2	Gram-positive rods	26	S	17	S
4.1	Gram-positive spore-forming rods	19	S	14	R
4.4	Gram-negative cocci	16	I	18	R

Based on the analysis of the data presented in Table 4 of the second experimental group, the following conclusions can be made: 0.06% chlorhexidine-based mouthwash demonstrated moderate bactericidal activity against all identified microorganisms. The effectiveness was noticeably lower than in the first experimental group. The average diameter of the inhibition zone was 16.57 mm. The essential oil-based mouthwash showed less bactericidal activity against all microorganisms in the second group, with an average inhibition zone diameter of 7 mm.

Khalij Libya Journal of Dental and Medical Research. 2026;10(2):175-184
https://doi.org/10.47705/kjdmr.26215
eISSN:2708-888X

Table 4. Bactericidal activity of 0.06% chlorhexidine-based mouthwash and essential oil-based mouthwash against identified microorganisms of the 2nd group.

2nd Experimental Group		0.06% Chlorhexidine-based mouthwash		Essential oil-based mouthwash	
		Diameter of the growth inhibition zone, mm			
Sample No.	Microorganisms	R ≤ 14, I = 15-16, S ≥ 17			
2.1	Gram-positive rods	19	S	0	R
2.3	Gram-positive cocci	14	R	0	R
10.1	Gram-negative cocci	16	I	9	R
10.2	Gram-negative spore-forming rods	14	R	7	R
13.3	Gram-negative rods	18	S	12	R
14.3	Gram-positive spore-forming rods	21	S	10	R
15.1	Candida	14	R	11	R

The 0.06% chlorhexidine-based mouthwash demonstrated moderate bactericidal activity against all identified microorganisms in the third group, as tabulated in (Table 5), including Gram-positive cocci, Gram-negative cocci, and Gram-negative rods, respectively. Further, the average diameter of the inhibition zone was 19.67 mm.

Table 5. Bactericidal activity of 0.06% chlorhexidine-based mouthwash and essential oil-based mouthwash against identified microorganisms of the 3rd group.

3rd Experimental Group		0.06% Chlorhexidine-based mouthwash		Essential oil-based mouthwash	
		Diameter of the growth inhibition zone, mm			
Sample No.	Microorganisms	R ≤ 14, I = 15-16, S ≥ 17			
6.2	Gram-positive cocci	21	S	0	R
8.3	Gram-negative cocci	18	S	18	S
9.1	Gram-negative rods	20	S	14	R

Discussion

The impact of orthodontic treatment on the prevalence of periodontitis remains an active topic for scientific investigations [11]. The relationship between orthodontic therapy and periodontal health is complex and often considered bidirectional [12]. On one hand, orthodontic treatment can improve periodontal conditions by aligning teeth and eliminating occlusal trauma, thereby facilitating more effective oral hygiene. On the other hand, orthodontic appliances may contribute to increasing the accumulation of supra-gingival biofilm and potentially compromise periodontal health [11-12]. Therefore, the key objective in orthodontic therapy is to achieve tooth movement without adversely affecting the integrity of periodontal tissues [11].

It is well known that the principal plaque-induced periodontal diseases include gingivitis and periodontitis. Gingivitis is an inflammatory condition that does not result in tissue destruction and is generally reversible with adequate plaque control. In contrast, periodontitis is influenced by both genetic and environmental predisposing factors, which may persist even after restoration of oral hygiene, leading to irreversible attachment loss and eventual tooth loss due to chronic inflammation initiated by periodontopathogenic bacteria [11-13].

Furthermore, multiple systematic reviews have demonstrated deterioration in clinical periodontal parameters during orthodontic treatment, including the oral hygiene index (OHI), bleeding on probing (BOP), attachment loss, and development of periodontal pockets or gingival recession. The extent of these changes varies depending on treatment duration and modality, with partial reversibility observed after treatment completion in some cases [11-14].

In addition to periodontal implications, plaque accumulation in the presence of orthodontic appliances is associated with an increased risk of dental caries and with qualitative and quantitative shifts in the oral microbiota. These changes may contribute to infections with potential systemic implications [15-16].

Consequently, orthodontic patients may present a higher overall risk of oral and systemic pathologies compared to individuals not undergoing orthodontic treatment [16].

Given these increased microbial and periodontal risks, chemical plaque control measures are often considered as adjunctive strategies. Mouth rinses have been widely used as antibacterial agents to reduce microbial load and prevent microbial spread. Several active substances are recommended for this purpose. Chemical agents in mouth rinses should effectively modulate the oral microbiota by selectively targeting pathogenic species while preserving the normal flora [17]. Typically, the oral antiseptics consist of combinations of active substances in solution and can be classified into several groups, including cationic surfactants, bisbiguanide derivatives, bispyridinamines, quaternary ammonium compounds, iodine-based compounds, phenolic agents, alcohols, and various organic mixtures [14-17-19].

Chlorhexidine (CHX) remains the most widely used oral antiseptic due to its high substantively and strong binding affinity to oral tissues [20]. However, its status as the "gold standard," it is associated with adverse effects; including staining of teeth and oral mucosa [21]. Current research focuses on combining chlorhexidine with anti-discoloration systems designed to reduce staining without compromising antimicrobial efficacy [22-23].

The findings of the present study should be interpreted in the context of the growing body of evidence regarding the use of CHX mouthwash in orthodontic patients. In the context of existing literature evaluating CHX mouthwash in orthodontic patients, Karamani *et al.* reported that CHX mouth rinses effectively reduce plaque accumulation and gingival inflammation compared with untreated controls; however, their meta-analysis also demonstrated that CHX is not consistently superior to alternative mouth rinses such as herbal, probiotic, or propolis-based formulations, with no statistically significant differences observed for gingival index outcomes in some comparisons [24]. This is consistent with the present study, where 0.06% CHX exhibited the highest antimicrobial activity, yet its effectiveness varied across experimental groups and was not uniformly superior under all conditions. Additional evidence by Amini *et al.* further expands the understanding of CHX effects, showing that while orthodontic treatment alone produces minimal changes in oral mucosal cellular indices, the adjunctive use of CHX may significantly alter nuclear parameters associated with mucosal cell vitality [24-25]. These findings suggest that the CHX, highly effective microbiologically, may also exert biological effects on oral tissues that should be considered in clinical use. Similarly, Andrucioli *et al.* demonstrated that the CHX mouthwash leads to significant improvements in plaque, gingival, and gingival bleeding indices when used twice weekly over a 30-day period; however, these benefits were accompanied by undesirable side effects, including mild to pronounced extrinsic tooth staining over time [24-25].

More recently, Tomljanovic *et al.* investigated the CHX effect on subgingival bacterial endotoxin activity and periodontal status in patients treated with metal and non-metal orthodontic appliances [26]. Their results showed that the use of CHX did not significantly influence endotoxin activity, periodontal status, or plaque pH when compared with standard oral hygiene measures [26]. Additionally, gingival inflammation was found to be associated primarily with endotoxin levels, plaque accumulation, and patient age rather than appliance type or CHX use [26]. These findings suggest that although CHX can contribute to microbial control, other biological and behavioural factors may play a more important role in determining periodontal outcomes during orthodontic treatment.

Taken together, the available evidence indicates that the CHX remains one of the most effective antimicrobial agents for reducing oral microbial load and controlling gingival inflammation in orthodontic patients [27]. This conclusion is supported by the present study, in which CHX consistently demonstrated the greatest bactericidal activity among the tested mouthwashes. However, the literature also demonstrates that its clinical superiority over alternative formulations is not universal and that its use may be associated with undesirable effects, including tooth staining and potential alterations in oral mucosal cell vitality.

The study of essential oil-based mouthwashes is complicated by variability in composition and difficulties in isolating active constituents, as their chemical profile is influenced by multiple environmental and processing factors [28]. In dentistry, essential oil-based mouthwashes are used in preoperative rinses, periodontal therapy, postoperative care and routine oral hygiene [28]. However, compared to antiseptics such as CHX, essential oils demonstrate lower and less consistent bactericidal activity with greater variability in antimicrobial efficacy depending on formulation and concentration. While they may provide adjunctive benefits in plaque control, their overall clinical effectiveness remains less predictable than that of conventional chemical antiseptics [28-29].

The comparatively lower antimicrobial activity observed for the essential oil-based mouthwash in the present study is consistent with previous reports in the literature [28]. Although essential oil-based mouthwashes are widely used in dentistry for preoperative rinsing, periodontal therapy, postoperative care, and routine oral hygiene maintenance, their antimicrobial performance is influenced by considerable variability in composition [28-30]. The study of these products is complicated by the difficulty of

https://doi.org/10.47705/kjdmr.26215
eISSN:2708-888X

identifying and standardising their active constituents, as the chemical profile of essential oils can be affected by plant species, geographic origin, environmental conditions, harvesting methods, and extraction processes [28-31]. Consequently, the antimicrobial efficacy of essential oil-based mouthwashes may vary substantially between formulations. Compared with CHX, essential oils generally exhibit lower and less predictable bactericidal activity, which may explain the smaller inhibition zones observed in the present study [31]. While essential oil-based mouthwashes may contribute to plaque control and oral hygiene maintenance, their clinical effectiveness appears to be more formulation-dependent and less consistent than that of conventional antiseptics such as chlorhexidine [31]. These observations support the findings of the present investigation, in which the essential oil-based mouthwash demonstrated substantially lower antimicrobial activity than the chlorhexidine-based formulation across the tested microbial groups.

Conclusion

Within the limitations of this study, the 0.06% chlorhexidine-based mouthwash demonstrated superior antimicrobial activity compared with the chlorhexidine-free essential oil-based mouthwash across all experimental groups. The CHX effectively inhibited the growth of Gram-positive and Gram-negative cocci and rods, spore-forming bacteria, and Candida species, producing mean inhibition zone diameters of 20.80 mm, 16.57 mm, and 19.67 mm for Groups 1, 2, and 3, respectively. In contrast, the essential oil-based mouthwash demonstrated limited antimicrobial effectiveness, with predominantly resistant responses and substantially smaller inhibition zones across all tested microbial groups.

Future studies should include larger patient populations and longer observation periods to better evaluate the relationship between orthodontic appliances, oral microbiota, and antimicrobial agents. Further investigations should assess a wider range of mouthwash formulations and active ingredients, including antiseptic, herbal, probiotic, and essential oil-based compounds, to identify alternatives with efficacy comparable to CHX and their adverse effects.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

References

1. Ren X, et al. The efficacy of mouthwashes on oral microorganisms and gingivitis in patients undergoing orthodontic treatment: a systematic review and meta-analysis. BMC Oral Health. 2023;23(1):204.
2. Mei L, et al. Factors affecting dental biofilm in patients wearing fixed orthodontic appliances. Prog Orthod. 2017;18(1):4.
3. Krishnan V, et al. Gingiva and orthodontic treatment. Semin Orthod. 2007;13(4):257-271.
4. Ren Y, et al. Orthodontic treatment with fixed appliances and biofilm formation-a potential public health threat?. Clin Oral Investig. 2014;18(7):1711-1718.
5. Contaldo M, et al. Early childhood oral health impact scale (ECOHIS): literature review and Italian validation. Int J Dent Hyg. 2020;18(4):396-402.
6. Deus FP, Ouanounou A. Chlorhexidine in dentistry: pharmacology, uses, and adverse effects. Int Dent J. 2022;72(3):269-277.
7. Straub H, et al. Bacterial adhesion on soft materials: Passive physicochemical interactions or active bacterial mechanosensing?. Adv Healthc Mater. 2019;8(5):1801323.
8. Pan S, et al. Prevalence of genotypes of porphyromonas gingivalis in adolescent orthodontic patients. PLoS One. 2017;12(11):e0188420.
9. Ingham CJ, et al. The micro-Petri dish, a million-well growth chip for the culture and high-throughput screening of microorganisms. Proc Natl Acad Sci U S A. 2007;104(46):18217-18222.
10. Hudzicki J. Kirby-Bauer disk diffusion susceptibility test protocol. Am Soc Microbiol. 2009;15(1):1-23.
11. Viljoen A, et al. How microbes use force to control adhesion. J Bacteriol. 2020;202(12):e00125-20.
12. Rubnikovich S, et al. Effectiveness of antibacterial therapy following the implantation of orthodontic temporary anchorage devices. Stomatol Minsk. 2022;(2):8-15.
13. Paluch E, et al. Prevention of biofilm formation by quorum quenching. Appl Microbiol Biotechnol. 2020;104(5):1871-1881.
14. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. J Oral Maxillofac Pathol. 2019;23(1):122-128.
15. Jockel-Schneider Y, et al. Impact of 0.1% octenidine mouthwash on plaque re-growth in healthy adults: a multi-center phase 3 randomized clinical trial. Clin Oral Investig. 2021;25(7):4681-4689.
16. Kreve S, Dos Reis AC. Bacterial adhesion to biomaterials: What regulates this attachment? A review. Jpn Dent Sci Rev. 2021;57:85-96.
17. Socransky SS, et al. Microbial complexes in subgingival plaque. J Clin Periodontol. 1988;25(2):134-144.
18. Contaldo M, et al. The oral microbiota changes in orthodontic patients and effects on oral health: an overview. J Clin Med. 2021;10(4):780.
19. Guest RL, et al. YejM modulates activity of the YciM/FtsH protease complex to prevent lethal accumulation of lipopolysaccharide. mBio. 2020;11(2):e00645-20.

20. Kamio Y, Nikaido H. Outer membrane of Salmonella typhimurium: accessibility of phospholipid head groups to phospholipase and cyanogen bromide activated dextran in the external medium. Biochemistry. 1976;15(12):2561-2570.
21. Santonocito S, Polizzi A. Oral microbiota changes during orthodontic treatment. Front Biosci (Elite Ed). 2022;14(3):19.
22. Hummels KR, et al. Coordination of bacterial cell wall and outer membrane biosynthesis. Nature. 2023;615(7951):300-304.
23. Ramsey JT, et al. Essential oils and health. Yale J Biol Med. 2020;93(2):291-305.
24. Karamani I, et al. Chlorhexidine mouthwash for gingivitis control in orthodontic patients: a systematic review and meta-analysis. Oral Health Prev Dent. 2022;20(1):317-326.
25. Amini F, et al. Effects of fixed orthodontic treatment with and without chlorhexidine mouthwash on vitality of oral mucosal cells reflected by cell nuclear indexes: A preliminary 3-phase before-after clinical trial. Int Orthod. 2023;21(1):100722.
26. Tomljanovic M, et al. Effect of chlorhexidine mouthwash on subgingival bacterial endotoxin activity and periodontal status during treatment with metal and non-metal fixed orthodontic appliance: a randomized controlled trial. Clin Oral Investig. 2025;29(8):387.
27. Andrucioli MD, et al. Effect of chlorhexidine mouthwashes on periodontal parameters and extrinsic tooth staining in orthodontic patients. Am J Orthod Dentofacial Orthop. 2023;164(6):855-861.
28. Kanagalingam J, et al. Practical use of povidone-iodine antiseptic in the maintenance of oral health and in the prevention and treatment of common oropharyngeal infections. Int J Clin Pract. 2015;69(11):1247-1256.
29. Panagiotou A, et al. Role of essential oil-based mouthwashes in controlling gingivitis in patients undergoing fixed orthodontic treatment. A review of clinical trials. Int J Environ Res Public Health. 2021;18(20):10825.
30. Dagli N, et al. Essential oils, their therapeutic properties, and implication in dentistry: A review. J Int Soc Prev Community Dent. 2015;5(5):335-340.
31. Wińska K, et al. Essential oils as antimicrobial agents-myth or real alternative?. Molecules. 2019;24(11):2130.