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Özgün Araştırma Makalesi

The Effect of Hydrogen Peroxide/Colloidal-Ag on *Staphylococcus aureus*: A Pilot Study

Hidrojen Peroksit/Kolloidal Gümüş'ün Staphylococcus aureus Üzerindeki Etkisi: Pilot Çalışma

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ABSTRACT

Aim: To determine the distribution of Staphylococcus aureus (*S. aureus*) aerosols in the immediate environment of dental operators during routine dental treatment and to investigate the reduction in *S. aureus* colonization using 0.5% hydrogen peroxide/colloidal silver disinfectant were aimed.

Material and Method: The study was divided into two groups. Sterile distilled water was used in Group A (n = 90 Petri plates). Sterile distilled water with hydrogen peroxide/colloidal silver was used in Group B (n = 90 Petri plates). The plates were set up in two directions on the head phantom corresponding to the 6 and 9 o'clock positions. Three petri plates were placed side by side up to 50 cm, 100 cm and 200 cm distances, and the experimental design were proceeded by contaminating with *S. aureus* (ATCC 29213). Mann Whitney U test was used for comparing to independent samples.

Results: When compared to Group A, *S. aureus* grown colonies on agar plates in Group B was significantly decreased in directions corresponding to the 6 and 9 o'clock positions to 50 cm, 100 cm, and 200 cm distances (p = 0.00).

Conclusion: Hydrogen peroxide/Colloidal-Ag can safely be used as a supportive precaution against *S. aureus*.

Keywords: Contamination; Hydrogen peroxide/colloidal silver; Staphylococcus aureus

ÖZET

Amaç: Mevcut çalışmanın amacı, rutin diş tedavisi sırasında diş hekimlerinin yakın çevresindeki Staphylococcus aureus (*S. aureus*) aerosollerinin dağılımını belirlemeyi ve hidrojen peroksit/kolloidal gümüş dezenfektanı kullanarak *S. aureus* kolonizasyonundaki azalmayı araştırmaktır.

Gereç ve Yöntem: Çalışma iki gruba ayrılmıştır. Grup A'da (n=90 petri kabı) steril distile su, Grup B'de (n = 90 petri kabı) hidrojen peroksit/kolloidal gümüş ilaveli steril distile su kullanılmıştır. Petri kapları, saat 6 ve 9 pozisyonlarına karşılık gelen bir kafa fantomu üzerine iki yönde ayrı ayrı yerleştirilmiştir. 50 cm, 100 cm ve 200 cm mesafelere kadar üç petri kabı yan yana yerleştirilip *S. aureus* (ATCC 29213) solüsyonu ile kontamine edilmiştir. Veri analizlerinde Mann Whitney U testi kullanılmıştır.

Bulgular: Grup B'de *S. aureus* bakteri sayısı Grup A'ya göre 50 cm, 100 cm ve 200 cm mesafelere kadar saat 6 ve 9 pozisyonlarına karşılık gelen yönlerde istatistiksel olarak anlamlı derecede azalmıştır (p = 0.00).

Sonuç: Hidrojen Peroksit/Kolloidal-Ag dental işlemlerde *S. aureus*'a karşı destekleyici önlem olarak güvenle kullanılabilir.

Anahtar Kelimeler: Hidrojen peroksit/kolloidal gümüş; Kontaminasyon; Staphylococcus aureus

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INTRODUCTION

Bio-aerosols are made of particles/droplets with live microorganisms. The high-speed dental handpiece, ultrasonic scaler, air polisher, and air/water syringe used in dental procedures may spread bioaerosols efficiently. Aerosolized microorganisms, which can be entrained or suspended in the air for considerable periods generated by these dental instruments, could spread to around 200 cm. Healthcare workers run a greater risk of acquiring respiratory pathogens since many dental procedures are known to aerosolize mouth and respiratory tract secretions in dental settings.[1-3]

Bacterial aerosols, predominantly Staphylococcus aureus (*S. aureus*), a resident strain of oral flora, have been formed during many dental procedures.[4] *S. aureus* transmission through the air or person to person has been confirmed in higher frequency in the mouth and nose of healthy individuals.[5] It can transmit through the saliva, blood, and aerosols to the practitioner, personnel, and other patients, causing cross-contamination during dental settings[6-10] and causing skin infections, septicemia, pneumonia, osteomyelitis, an abscess.[11] *S. aureus* has crucial importance to be resistant to heat and many chemicals and to be able to remain in the dental environment as well. Dental unit waterlines (DUWs) have also been included as potential bacterial reservoirs.[4] DUWs biofilm, conceptually similar to the oral microbiome indicates that contamination from patient-derived bacteria can occur from the functional end of the line.[12] Identifying environmental reservoirs of both Methicillin-sensitive *S. aureus* (MSSA) and Methicillin-resistant *S. aureus* (MRSA) in society is critical to controlling the spreading of staphylococcal infections. Contamination of DUWs by MSSA strains and MRSA strains is crucial as it is considered a causative agent for community-acquired infections that are elevated very much.[13,14]

There is a broad series of products used in DUWs, which can reduce the microbial burden and eliminate biofilm formation of the DUWs. In addition, hydrogen peroxide-based disinfectant is also used to reduce total viable counts as the definitive measure of total microbial contamination of the water passing through the DUWs. Using hydrogen peroxide colloidal-Ag-based disinfectant in DUWs reduces dental units' microbial counts to less than 200 CFU/mL (colony forming units), which is recommended as a standard microbial count for water samples by American Dental Association (ADA).[13,15] Bio-aerosol generation procedures should be judiciously controlled. A dental team should use precautionary measures such as hand hygiene procedures, clothes, disposable gloves, face masks, eyeglasses, visor shields, and disinfectants in DUWs to reduce cross-contamination and avoid dissemination to the clinical environment.[1,15]

The current study aimed to determine the distribution of *S. aureus* aerosols in close vicinity of dental operators during routine dental treatment. Besides that, the present study aims to investigate the reduction in the colonization of *S. aureus* by using hydrogen peroxide/Colloidal-Ag disinfectant in DUWs.

MATERIAL AND METHOD

One dental chair unit (DCU) and three petri plates per the chosen distances for each of the chosen directions were used in the study. A 3.40 m length x 4.30 m width x 2.60 m height (38.01 m^3 volume) operating room was used for the experimental setting. The room was disinfected using fog with hydrogen peroxide Colloidal-Ag disinfectant (1% Silveroxy-A, Anitek, Turkey) for 15 minutes to reduce the aerosol concentration before each experimental setting. The tap water system was shut down due to using the in-built bottle system of the DCU.[13] The DCU has been treated to remove biofilm or reduce planktonic bacterial contamination before the experiments using hydrogen peroxide/Colloidal-Ag.

The study was divided into two groups. Only sterile distilled water was used in Group A, which was designed as the control group (n = 90 Petri plates (n, the total number of sampling plates is 90)). Sterile distilled water with the 500 ppm hydrogen peroxide Colloidal-Ag was used in Group B, serving as the study group (n = 90 Petri plates).

Before the experiment, the high-speed dental handpiece and air-water syringe were flushed for 2 minutes using sterile distilled water. The sterile swab samples from the head phantom's jaws were collected.

The study was carried out on a head phantom fitted, simulating the operational position on the DCU. From the headrest of the DCU, the wooded batten was fitted on a plane 90 cm above the floor from a point 10 cm below the head phantom's jaw. Petri plates were set up separately in two directions corresponding to the 6 and 9 o'clock positions in the experimental setting. Three Petri plates were placed side by side on the wooded batten up to 50 cm, 100 cm, and 200 cm distances for each experimental setting.

The mouth rinse consisted of hydrogen peroxide Colloidal-Ag disinfectant (concentration of 1%), which was applied for 5 minutes, and then sterile distilled water was used for 1 minute before the experiments. The standard strain of *S. aureus* ATCC 29213 (American Type Culture Collection, Manassas, VA) was used in this study. *S. aureus* was grown on blood agar medium (Merck, Germany) at 370C aerobically for 24-48 hours, and the freshly grown colonies were harvested to prepare the bacterial suspension in the sterilized distilled water with a concentration of 1.5x108 colony-forming unit (CFU)/mL adjusted according to 0.5 McFarland standard turbidometrically. Besides, the bacterial suspension was also measured spectrophotometrically (OD:0.600 nm), and the head phantom's jaws were contaminated by *S. aureus* suspension for 5 minutes in each direction corresponding to the 6 and 9 o'clock positions before each experiment.

Aerosol generated in dental patients' mouths was simulated by applying a high-speed dental handpiece with water spraying, which was placed on the lingual side and then on the buccal side of the mandibular central incisor teeth for one minute. In addition, dental suction was activated during aerosol generation. Petri plates were exposed for 120 seconds to perform each experiment.

The same procedures were also performed for the study group. After the experimental processes were completed, all the Petri plates were brought to the microbiology laboratory and put into the incubator for 24-48 hours at 37^0C under aerobic conditions. After the incubation period was completed, the plates were evaluated for bacterial growth, and the grown colonies were counted. Those high numbers were counted by using a magnifier and adjusting a one mm (1x1mm) scale put at the back side of the plate under a Stereo Zoom microscope (Nikon Co., Ltd., Tokyo, Japan). Then the data was recorded for each plate as CFU/mL. The data from ten-experimental settings for each direction was assessed for the efficacy of hydrogen peroxide/colloidal-Ag disinfectant in reducing bio-aerosol generation.

IBM SPSS Statistics 25 package is used for data analyses. In this study, descriptive statistics; Frequencies, percentages, min, max, mean, median, and standard deviation, are used to describe the data at hand. Since the distribution was not normal, non-parametric tests were used. Statistical analysis was performed by the Mann-Whitney U test for comparing independent samples. Mean rank and p values are listed in the tables. Meaningful statistical significance was assumed when $p<0.05$.

RESULTS

Total bacterial counts at 50 cm, 100 cm, and 200 cm in Group A and Group B within the 6 o'clock and 9 o'clock positions are given in Table 1. Bacterial counts are expressed as colony-forming units (CFU).

The descriptive statistical analysis of the results is presented in Table 2.

The results showed there are statistically significant differences in Group A and Group B bacterial counts at 50 cm, 100 cm, and 200 cm distances in two directions corresponding to the 6 and 9 o'clock positions ($p<0.05$) (Table 3).

Table 1. Total counts of S. aureus in the study groups

	Total bacterial count (CFU/mL)					
	Group A			Group B		
	50 cm	**100 cm**	**200 cm**	**50 cm**	**100 cm**	**200 cm**
6 o'clock	152674	6484	388	1219	385	203
9 o'clock	906	353	352	355	103	65

Table 2. Descriptive statistical analysis of the study groups

		Group A (CFU/mL)					Group B (CFU/mL)				
		Min	Max	Mean	Median	Std Deviation	Min	Max	Mean	Median	Std Deviation
6 o'clock	50 cm	41	120000	5089.13	482	21814.11	8	122	40.63	30.50	31.47
	100 cm	2	430	70.93	35	106.18	3	46	12.83	9	10.46
	200 cm	3	38	12.93	13.00	7.87	0	15	6.77	5.50	4.26
9 o'clock	50 cm	2	149	30.20	13.50	37.58	1	55	11.83	6.50	15.45
	100 cm	2	25	11.77	10.50	5.08	0	13	3.43	3.00	2.69
	200 cm	3	26	11.73	11.00	5.94	0	5	2.17	2.00	1.49

Table 3. The differences between Group A and Group B with Mann Whitney U test

		Group A Mean Rank	Group B Mean Rank	Group A – Group B (CFU/mL) p values
6 o'clock	50 cm	45.07	15.93	0.00
	100 cm	40.95	20.05	0.00
	200 cm	38.42	22.58	0.00
9 o'clock	50 cm	37.22	23.78	0.00
	100 cm	43.43	17.57	0.00
	200 cm	44.72	16.28	0.00

DISCUSSION

The oral cavity harbors more than 700 bacterial species, including *S. aureus*.[16,17] The oral cavity represents a significant region of *S. aureus* spreading. The aerosols and splatter generated during dental procedures have the potential to spread the infection through the air or person to person, causing cross-infection during dental procedures and becoming an important health problem.[18]

This study aimed to improve our knowledge and advance the understanding of the effect of H_2O_2/ Colloidal-Ag on *S. aureus*. Although various barrier procedures, dental staff can be exposed to notable splatter and aerosol dispersion. Total bacterial counts in two different directions corresponding to the 6 and 9 o'clock positions at 50 cm, 100 cm, and 200 cm in Group A and Group B for 90 Petri plates are presented in Table 1. While the maximum number of bacteria at 50 cm, which corresponds to the 6 o'clock position of group A samples, was 152674 CFU/mL, the total number of bacteria in group B, in which we used hydrogen peroxide colloidal silver, decreased to 1219 CFU/mL in the same direction and distance. In addition, the minimum total bacterial counts at 200 cm in Group A and Group B within the 9 o'clock position were 352 CFU/mL, 65 CFU/mL, respectively.

While performing dental procedures in the anterior region of the mandible, the highest aerosol splash was observed at 50 cm in 6 o'clock position. This study showed that the 0.5% H_2O_2/Colloidal-Ag could reduce *S. aureus* aerosols contamination in the operator position and in front of a patient.

Hydrogen peroxide disinfectants can destroy both the biofilm matrix and the bacterial cells within this biofilm, making them a preferable anti-biofilm agent. It had effective bactericidal activity against *S. aureus* biofilms, which may be widespread in a dental office, consistent with the previous studies.[19,20]

On the contrary to the bacteriostatic or mild bactericidal activity obtained by using each agent alone, the use of the synergistic antibacterial activity of the silver nanoparticles and hydrogen peroxide combination (about 0.03%), even at relatively low concentrations, resulted in the complete eradication of the *S. aureus*.[19] We also used a hydrogen peroxide/ colloidal silver to reduce *S. aureus* colonization in the current study. As reported in the study by Lineback *et al*., a statistically significant decrease in *S. aureus* colonization was observed in the study group. The produced aerosols can remain in the air for hours.[2] Bacteria can adapt themselves to a sublethal stress and become more resistant to following

implementations of the same stress (homologous resistance).[21] Besides, *S. aureus*, a catalase-positive bacterium, catalyzes hydrogen peroxide, forming O_2 and H_2O[22] to avoid the harmful effect of H_2O_2. Besides, under the hydrogen peroxide exposure, *S. aureus* can tolerate this switching to the generation of small-colony variants with an enhanced catalase production.[23]

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In conclusion, the aerosols and splatter produced during dental procedures have a crucial potential to spread the infection to dental staff and other people. Dental staff appears to use routinely personal protective barriers such as masks, gloves, and safety glasses, which decrease contact with bacterial aerosols and splatters. The dental personnel should not trust only a single precautionary procedure. It is possible to minimize the risk posed by dental aerosols with relatively straightforward and inexpensive supportive precautions such as hydrogen peroxide/ silver ions disinfectant that is particularly efficient at reducing the heterotrophic bacteria and eradicating the biofilm in DUWs. According to the results of this study, H_2O_2/Colloidal-Ag (0.5%) can be used to take safe and supportive precaution against *S. aureus* spread at the chosen positions.

Since the characteristic properties of *S. aureus*, further studies which have extended groups and directions can be performed for the best results.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

No ethical approval for this study.

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