Chulalongkorn University Dental Journal

Volume 26 | Issue 2 Article 5

2003-05-01

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ว ทันต จุฬาฯ 2546;26:137-45

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Abstract

Objective This study was designed to assess the microbiological quality of the water used in the dental clinic of Naresuan University (NU).

Materials and methods By means of standard plate counts with the use of violet red bile agar, microorganisms in water samples gathered from dental units before and after dental procedures were cultivated and examined at light microscopic level.

Results The observed Gram-negative bacilli without spore formation were regarded as total coliforms. Water samples from plastic bottles attached to the dental units and from air-water syringe tips revealed few, if any, colony-forming units (cfu) of total coliforms, while those from the cups for patients to rinse their mouth showed no cfu. Compared to those from other sources, the samples from high-speed handpieces possessed significantly more cfu ($p=0.043$). Time-related significant differences in microbial number were also detected in the samples gathered from the handpieces ($p < 0.05$).

Conclusion Despite an existence of total coliforms, the water used during treatment in NU's dental clinic fulfilled the standard requirement of American Dental Association. Nevertheless, it is necessary that there be strict quality programs, including regular water monitoring, for microbiological analyses.

(CU Dent J 2003;26:137-45)

Key words: *dental unit; Microbiological quality; total coliforms; water*

water sample into 20 mL of the prepared agar solution (40°C, pH 7.4). It was then covered by a sterilized glass lid and vibrated by a hand on a flat table for 30 sec to obtain a thorough mixture. The plate was left at the room temperature for 90 min and then incubated in an aerobic incubator (WTB binder: Labfocus, Bangkok, Thailand) at 37°C for 24 hours. Colony-forming units (cfu) were inspected and counted with naked eyes.

For final visualization of the microbial morphology, all colonies were processed as suggested by Bryan et al.[6]. Briefly, each colony was smeared on a glass slide with an inoculating loop, air-dried gently, and then heat-fixed by a flame. The smear was stained for 1 min with 0.1% crystal violet in distilled water (DW), followed by a 1-min treatment with 0.33% iodine in DW. After washing with tap water, it was decolorized in 95% ethanol for 15 sec and then counterstained for 1 min with 0.1% safranin in DW. All stained microbes were examined under a bright-field light microscope (Leica-DMLB: Leica, Nussloch, Germany).

Statistical analyses

All data were analyzed using SPSS for Window version 10.0 statistical package. The level of significance was set at 0.05.

Results

The samples obtained from autoclaved water and handpiece lubricant showed no visible microbial colonies in the agar plates.

All observable microbial colonies were pale pink in color and round in shape, with a diameter of approximately 1-2 cm (Figure 1). Light microscopic observation of the smeared and stained colonies revealed only red microorganisms possessing a rod shape, about 2 μm long, which were regarded as total coliforms (Figure 2). No spore formation was recognizable.

From S1 at both collection periods, no cfu of the total coliforms was visible in agar plates (Table 1).

Table Colony-forming units per milliliter of water samples obtained from each source before and after dental procedures.

Dental unit number	Stainless steel cup (S1)		Plastic bottle (S2)		Air-water syringe tip (S3)		Dental handpiece (S4)	
	Before	After	Before	After	Before	After	Before	After
	0	0	0	0	0	0	125	11
2	0	0	0	0	34	0	132	115
	0	0	0	0	0	0	70	48
4	0	0	0	0	0	0	78	70
5	0	0	0	0	0	0	55	71
6	0	0	0	0	0	0	38	44
7	0	0	3	0	3	0	6	0
8	0	0	0	0	0	0	168	50
9	0	0	0	0	0	0	2	0
10	0	0	0	0	0	0	4	0
11	0	0	0	0	4	0	143	10
12	0	0	0	0	8	3	0	3
13	0	0	0	0		0	0	16
14	0	0	0	0	0	0	71	16
t-value	N/A		1.000		1.398		2.244	
Degree of freedom	13		13		13		13	
p-value	N/A		0.336*		0.185*		0.043**	

N/A: could not be determined by paired t-test.

* Non-significant difference between the samples obtained before and after the dental procedures.

** Significant difference between the samples obtained before and after the dental procedures.

From S2, cfu of total coliforms were found in only one sample that was obtained before the dental procedures (Table 1). Statistical analyses revealed no significant differences in the number of cfu between the samples collected before and after the dental procedures (t-value=1.000; df=13; p=0.336).

From S3, five samples gathered before and one sample harvested after the dental procedures revealed cfu of total coliforms (Table 1). Compared with those in the samples obtained after the dental procedures, cfu of total coliforms in the samples collected before the dental procedures were non-significantly larger in their number (t-value=1.398; df=13; p=0.185).

From S4, significantly more cfu of total coliforms were seen in the samples obtained before the dental procedures than those collected after the procedures (t-value=2.244; df=13; p=0.043). Statistical analyses showed that there was a significantly larger number of cfu of total coliforms in S4 than in S1, S2 and S3 (Table 2).

Table 2 Summary of p-values (paired t-test) calculated from and compared between colomy-forming units per milliliter of each water sample and the other.

Source		Stainless steel cup (S1)		Plastic bottle (S2)		Air-water syringe tip (S3)		Dental handpiece (S4)	
		Before	After	Before	After	Before	After	Before	After
Stainless steel cup (S1)	Before			0.336*	N/A	0.164*	0.336*	0.001**	0.004**
	After			0.336*	N/A	0.164*	0.336*	0.001**	0.004**
Plastic bottle (S2)	Before	0.336*	N/A			0.191*	1.000*	0.002**	0.005**
	After	0.336*	N/A			0.164*	0.336*	0.001**	0.004**
Air-water syringe by (S3)	Before	0.164*	0.336*	0.191*	1.000*			0.002**	0.004**
	After	0.164*	0.336*	0.164*	0.336*			0.002**	0.005**
Dental handpiece (S4)	Before	0.001**	0.004**	0.002**	0.005**	0.002**	0.004**		
	After	0.001**	0.004**	0.001**	0.004**	0.002**	0.005**		

N/A could not be determined.

* Non-significant difference.

** Significant difference.

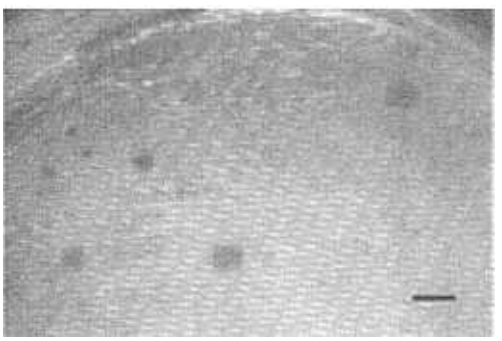

Figure 1 Colony forming units observed in the violet red bile agar media containing water samples. Each colony is pink in color and round in shape, with a diameter of approximately 1-2 cm. (x1; scale bar: 3 cm).

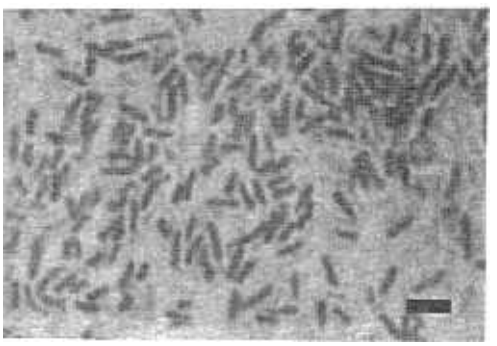

Figure 2 Light micrograph showing morphological structures of microorganisms existing in water samples. They are Gram-negative bacillus, about 2 μm long, and possess no spore formation. Gram's staining. (x100; scale bar: 3 μm).

Discussion

Although several methods are used for detecting bacteriological indicators, the agar plate count technique is widely conducted for microbiological analyses of drinking water quality[7,8]. Because of its specificity to total coliforms, violet red bile agar (VRBA) was used for an enumeration of total coliforms in solid and liquid samples[9,10]. The extent to which total coliforms are present in the water indicates the general quality of the water and the likelihood that it is contaminated[11]. These data suggest an effectiveness of the method used in this study, as well as a reliability of the close relationship between the investigated bacteria and the water utilized in the dental clinic.

Collection time-related differences in the number of bacterial growth

The samples obtained before dental procedures from dental handpieces showed a significantly larger number of total coliforms than those after the dental procedures. In addition, similar data were also seen in those gathered from plastic bottles and air-water syringe tips, despite their non-significant differences. This might be explainable by a static condition of the water within the bottles and dental units. For a prompt distribution of the water from a container to an air-water syringe tip or to a dental handpiece, plastic bottles filled with the filtered water were always attached to each dental unit. Water stagnation in the bottles might provide an opportunity for bacterial growth. Geldreich and Reasoner[12] reported that the 6-week no-flow period in a water container increased bacterial counts from 1,000- to 10,000-fold over densities, in association with overnight static periods. Although the water investigated in this study was not kept for such a long period, a complex design of dental equipment might result in the stagnation of water within each dental unit, subsequently causing an amplification of contaminating organisms. However, as the water was utilized during the day and the new one was then refilled, the stagnant circumstance of the water was decreased, resulting in an impediment of bacterial formation. The data implied a significance of regular replacement of the water in plastic containers attached to the dental units

Source-related differences in the number of bacterial growth

HSRI water was treated by an addition of chlorine into the water with a concentration of 2 parts per million (ppm). Chlorination has been reported to be highly effective in reducing the amount of wide-spectrum of bacteria in water[13]. In this study, no colonies of total coliforms were seen in the plates with water samples from stainless steel cups. It pointed out that HSRI water for dental patients to rinse their mouths fulfilled the microbiological standards proposed by WHO[1]. Nevertheless, various hazards of chlorine and its compounds for animal health were reported[14-16]. Consequently, the amount of chlorine in HSRI water needs a regular control to make it retain germicidal effects in a non-toxic level.

Apart from disinfection, HSRI water should be processed for removal of pyrogenous substances, water softening, removal of heavy metal and solvents, and removal of substances of bad taste. Prior to conduction of such courses, there should be strict considerations on needs of processing, all methods to be used by independent specialists, and a reasonable relation between operation costs and efficacy of processing system[17]. The mentioned information illustrated that representatives from all of NU's academic fields should collaborate on standardizing HSRI water quality.

Prior to transfer to the bottles, the water was passed through the device containing activated carbon and cation resin. The results from our investigation showed the contamination of total coliforms in one sample obtained from such bottles, in spite of its non-significantly different number from others. It has been known that activated carbon helps reduce bacteria[18] and that electropositve resin is suitable for the decrease of virus, organic compounds and inorganic elements[19-21]. Nonetheless, some non-effectiveness of those two filtering materials for the removal of microbial contaminants has been reported in the devices with an overnight period of nonuse[22,23]. For water filtration, the use of activated carbon over a period of 11 weeks has been revealed to have no significant effects on the number of bacteria present in the water, particularly total coliforms[24]. The data suggested that the water filtration materials be periodically changed to obtain their most effectiveness in the reduction of water contaminants.

Air-water syringe tip provides water and/or air to make the field visible and accessible. From our results, the water samples obtained from the syringe tips showed several colonies of total coliforms. Gaetti-Jardim et al.[25] have disclosed a possibility for the air-water syringe tips to serve as vehicles for transmission of oral pathogens. Sterilization of the tip or the entire syringe has been reported to be unable to completely eliminate some microorganisms, resulting in an existence of water contaminants[26]. Observed by scanning electron microscopy, the inner wall of plastic tubes supplying water to the air-water syringe is lined by bacteria-laden biofilms. Consequently, regular flushing the water lines with a biocide[26], as well as a weekly treatment with 5.25% sodium hypochlorite (diluted 1:10) and with 3 ppm chlorine in water[27], are recommended to obtain the standards proposed by ADA[3]. Taken together into consideration, it was suggested that the use of ADA guidelines[3] for sterilization of the syringe tips is needed, in association with the treatment of dental-unit water lines.

Even though the handpieces used in NU's dental clinic are autoclaved, some colonies of total coliforms were still observable. In addition, the data showed that the contamination of total coliforms in dental handpieces from both collection periods were significantly larger in their number than those in other sources. The results in this study have shown that the water kept in plastic bottles contained no total coliforms. It pointed out that the bacterial colonies seen in this source were derived from the water lines connecting between the plastic bottles and the dental handpieces. It has been reported that the inner part of water lines is lined with bacteria-laden biofilms[25]. An air pressure during the handpiece operation might slowly dislodge the biofilms and the microorganisms in the chamber of the unit, resulting in the bacterial contamination to the water. To help decrease the number of bacteria in water lines, the treatment processes for air-water syringe tips mentioned earlier have been claimed to be sufficient and practical[26,27]. Moreover, a continuous water flushing of more than four minutes is needed[28]. Using distilled water treated with or without some chemical agents has been reported to be effective in an improvement of water quality[29]. However, it is noteworthy for dental practitioners that the effluent water should be compatible with dental materials and be potentially free from toxic or carcinogenic materials.

ADA[3] recommends that all dental handpieces be cleaned by only heat sterilization. It was believed that flushing the water prior to use between each patient, along with sterilizing the dental handpiece by chemical substances, was enough to decrease the microbes. However, no significant difference in the reduction of cfu can be recognized after adding the handpiece to water line[30]. Though the handpieces used in NU's dental clinic were manufactured with an anti-retraction valve, the results gathered from them showed a significantly higher number of total coliforms than those from their controls and other sources. Regardless of the total coliforms that were originated from the plastic bottles, some oral fluids were possibly aspirated into the handpieces. Montebugnoli and Dolci[31] have shown that the dental handpieces internally equipped with the valve cause less, if any, aspirated fluid to go past and back into the handpieces. The discrepancy between our findings and theirs remains to be clarified, yet attributable to the different methods of investigation. Using the specific VRBA, we observed the cfu of total coliforms and considered their number the microbial contamination, while they simulated the global contaminants by means of a non-specific potassium bichromate dye aspirated to the dental handpieces. In addition, the differences in air pressure conveyed to the handpieces used in their study and ours, along with the subjacent variability in the size of the investigated contaminants, may result in the discrepancy. Despite the obtained results, the high-speed dental handpieces installed with an antiretraction valve and processed under a heat sterilization between each patient should be considered an essential component of standard procedures, whenever universal precautions are practiced in dentistry.

All of the results from this study indicated that the dental equipment and processes utilized in NU's dental clinic of NU were qualified, according to the standards systemized by ADA. Moreover, the data pointed out that the dental clinicians should follow all ADA recommendations and maintain an effectiveness of the devices used in their dental clinic.

Conclusion

From our study by means of standard plate counts, the water used during treatment in NU's dental clinic appears to meet the standard requirement determined by

ADA. The data also indicated that all sources of water supply to dental units could be the routes conveying total coliforms during dental procedures. The conduction of only one sterilizing technique to dental instruments and equipment may be insufficient for reducing microbial contaminants. In addition, the dental clinic should carry out strict quality programs, including regular water monitoring, for microbiological analyses.

Acknowledgements

The authors are grateful to Dr. Pairoje Sriaroon for his permission to our utilization of the dental units. We also thank Mr. Sittad Soypetcasem for his technical instructions, and Ms. Tussanee Meepayong, and Ms. Aree Thongthung for their assistance.

This work was supported by the research grant of Faculty of Dentistry, NU. Some parts of this study appear in the report submitted to the Faculty.

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คุณภาพทางจุลชีววิทยาของน้ำที่ใช้ในคลินิกทันตกรรม มหาวิทยาลัยนเรศวร

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บทคัดย่อ

วัตถุประสงค์ เพื่อประเมินคุณภาพทางจุลชีววิทยาของน้ำที่ใช้ในคลินิกทันตกรรม มหาวิทยาลัยนเรศวร

วัสดุและวิธีการ ทำการเก็บน้ำตัวอย่างจากเก้าอี้ทำฟันก่อนและหลังการปฏิบัติงานทางทันตกรรม แล้วนำไปเพาะเชื้อเพื่อหาโคลีฟอร์มทั้งหมดบน violet red bile agar ต่อจากนั้น นับจำนวนโคโลนีที่เกิดขึ้นในจานเพาะเชื้อและนำเชื้อไปตรวจสอบในระดับกล้องจุลทรรศน์แบบใช้แสง

ผลการศึกษา บาซิลลัสที่ย้อมติดสีแกรมลบและไม่มีการสร้างสปอร์นั้น มีลักษณะเป็นโคลีฟอร์มทั้งหมด น้ำตัวอย่างที่เก็บจากขวดพลาสติกซึ่งยึดติดกับเก้าอี้ทำฟันและจากที่เป่าลมและน้ำนั้น มีโคโลนีของโคลีฟอร์มทั้งหมดน้อยมาก แต่ไม่พบโคโลนีของโคลีฟอร์มทั้งหมดในน้ำตัวอย่างที่เก็บจากถ้วยน้ำสำหรับผู้ป่วยในการบ้วนปาก ส่วนน้ำตัวอย่างที่เก็บจากหัวกรอเร็ว มีจำนวนของโคโลนีของโคลีฟอร์มทั้งหมดมากกว่าที่เก็บจากแหล่งอื่น ๆ อย่างมีนัยสำคัญ ($p=0.043$) และจำนวนเชื้อที่พบในน้ำตัวอย่างที่เก็บจากหัวกรอก่อนและหลังการปฏิบัติงานทางทันตกรรมนั้น มีความแตกต่างกันอย่างมีนัยสำคัญ ($p < 0.05$).

สรุป แม้ว่าจะพบโคลีฟอร์มทั้งหมด แต่คุณภาพของน้ำที่ใช้ในคลินิกทันตกรรม มหาวิทยาลัยนเรศวร ก็อยู่ในระดับมาตรฐานที่กำหนดโดยสมาคมทันตแพทย์แห่งประเทศสหรัฐอเมริกา อย่างไรก็ตาม จำเป็นอย่างมากที่จะต้องมีโครงการควบคุมคุณภาพที่เข้มงวดและมีการกำกับดูแลน้ำที่ใช้อย่างสม่ำเสมอ สำหรับการวิเคราะห์ทางจุลชีววิทยา

(ว ทันต จุฬา 2546; 26:137-45)

คำสำคัญ: คุณภาพทางจุลชีววิทยา; จำนวนโคลีฟอร์ม; น้ำ; ยูนิตทำฟัน