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ASSESSMENT AND QUANTIFICATION OF THE MICROBIAL FLORA IN THE OUTPUT WATER OF TWO TYPES OF DENTAL CHAIRS: A COMPARATIVE STUDY

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Objectives: Dental Unit Waterlines (DUWL) have shown to be a perfect host for different pathogenic microorganisms. The purpose of this study was to analyze and compare the level of bacterial contamination in the output water of 2 types of DUWL.

Methods: Dental unit water samples from the air/water syringe of the A-dec (A-dec™ Performer 200, Newberg, USA) DUWL type and KaVo (ESTETICA™ E30/E70/E80 Vision, Kavo, Biberach, Germany) type were collected and analyzed for total aerobic flora, *Pseudomonas aeruginosa*, faecal coliforms, total coliforms, faecal *streptococci* and sulfite-reducing anaerobic flora.

Results: The bacteriological analysis of water samples shows the presence of bacterial contamination at high levels exceeding the standard safety guidelines of 100 CFU/mL set up by the American Dental Association (ADA) and accepted by the Centers for Disease Control and prevention (CDC) on the heterotrophic bacterial load. The data shows no statistically significant differences for all bacteriological parameters studied between the conventional A-dec DUWL type and the KaVo type that has an automated mode of decontamination.

Conclusions: Despite all the disinfecting solutions considered to eradicate the bacterial proliferation in DUWL, the problem remains one of the greatest challenges in modern dentistry. Practitioners and medical staff should not underestimate the harmful consequences of this bacterial growth, not only on the health of their patients but also on their own health.

Keywords: Bacterial infection; dental infection control; dentist-patient infection transmission

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ÉVALUATION ET QUANTIFICATION DE LA FLORE MICROBIENNE DANS LES EAUX DE SORTIE DE DEUX TYPES DE FAUTEUILS DENTAIRES : UNE ÉTUDE COMPARATIVE.

Objectifs: Les conduits d'eau des fauteuils dentaires (DUWL) se sont révélés être un hôte parfait pour différents micro-organismes pathogènes. Le but de cette étude est d'analyser et de comparer le niveau de contamination bactérienne dans les eaux de sortie de 2 types de DUWL.

Méthodes: Des échantillons d'eau provenant de la seringue air/eau de deux types de fauteuils dentaire A-dec (A-dec™ Performer 200, Newberg, USA) et KaVo (ESTETICA™ E30/E70/E80 Vision, Kavo, Biberach, Allemagne) ont été collectés et analysés pour la flore aérobie totale, Pseudomonas aeruginosa, les coliformes fécaux, les coliformes totaux, les streptocoques fécaux et la flore anaérobie sulfite-réductrice.

Résultats: L'analyse bactériologique des échantillons d'eau a montré la présence d'une contamination bactérienne à des niveaux élevés dépassant le seuil de sécurité standards de 100 CFU/mL accepté par l'American Dental Association (ADA) et par le Centers for Disease Control and Prevention (CDC). Les données ne montrent aucune différence statistiquement significative pour tous les paramètres bactériologiques étudiés entre le type A-dec conventionnel et le type KaVo doté d'un mode de décontamination automatisé.

Conclusions: Malgré toutes les solutions de désinfection envisagées pour éradiquer la prolifération bactérienne dans les DUWL, le problème reste l'un des plus grands défis de la dentisterie moderne. Les praticiens et le personnel médical ne doivent pas sous-estimer les conséquences nuisibles de cette prolifération bactérienne, non seulement sur la santé de leurs patients mais également sur leur propre santé.

Mots clés: Contrôle des infections dentaires; infection bactérienne; transmission de l'infection dentiste-patient.

Introduction

Bacterial contamination in the dental practice is one of the most challenging problems in modern dentistry. Bacteria are becoming more and more resistant to disinfecting agents and protocols [1], which poses a potentially significant risk of infections to both dental staff and patients; in particular those who are immunocompromised or suffer from serious health problems. In comparison to the Centers for Disease Control and prevention (CDC) and the American Dental Association (ADA) guidelines, many studies have recently shown that the water used in dental practice is contaminated by a bacterial niche at relatively high levels. The obtained levels exceeded by far the standards of 100 CFU/mL recommended by the ADA and accepted by the CDC of heterotrophic water bacteria [2-4]. The bacterial species found include gram-positive bacteria such as *Streptococcus mutans*, and gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter*, and *Legionella spp* [5]. These bacteria adhere and multiply inside the dental chair unit, form biofilms that stick to moist surfaces and can become very resistant to disinfecting agents and protocols. Some of them may be fatal if not treated within the first hours of infection.

The water used for spraying, cooling, ultrasonication procedures, and irrigation of the oral cavity can be supplied by either the municipal reservoir or the chair's bottle filled with distilled water. Water is transported into small tubules of several meters, referred to as the dental unit waterlines (DUWL). These tubules, often made of plastics such as polyvinyl chloride (PVC), are narrow and measure approximately 2-5 mm in diameter creating a suitable environment for bacterial growth and the formation of biofilm [6-7]. Almost without exception, surfaces within the aqueous and humid environments become colonized with microorganisms built up in a strong matrix-encapsulated biofilm maintained by the secretion of an adhesive. Once established, it becomes very difficult to eradicate the bacterial biofilms leading to microbial contamination of the DUWL [8-10]. Microbial cells can then be released into the water stream that eventually reaches the patient's mouth [11-12].

Bacterial biofilms are complex surface-attached communities of bacteria held together by self-produced polymer matrices mainly composed of polysaccharides, secreted proteins, and extracellular DNAs [13]. It is a living dynamic structure in perpetual reorganization that can be made up of a single bacterial species but, more often by a large number of species coexisting within the structure [11]. A recent study has shown that biofilms can form within 8 h after using a new dental chair for the first time [14]. Microorganisms growing in a biofilm are greatly adaptable to the natural environment, and are often highly resistant to adverse conditions such as disinfecting agents and protocols, antimicrobial agents, and antibiotics [15]. This resistance needs more research to be fully understood, yet it can be explained by the development of biofilm/attachment specific phenotypes and direct interactions among the exopolymer matrices, resulting in neutralizing the antibacterial effect of the disinfecting agents. Biofilms are difficult to eliminate even with the most advanced disinfecting agents and the fluids that circulate in the tubules will detach only its superficial fragments, which leads to a continuous contamination of the DUWL [16]. A study conducted by Neethu Salam *et al*. in 2017, found high levels of *Pseudomonas* in water samples from dental handpieces and 3-way syringes, exceeding the acceptable norms set up by the ADA and the CDC. Disinfection using chemicals such chlorhexidine, sodium hypochlorite, sterilox, and oxygenal reduced the bacterial count yet the biofilms persisted [17].

For the majority of the DUWLs with an unacceptable water standard, this is due to neglect or incorrect practices for water-cleaning procedures. In fact, it is recommended to flush all the rotary instruments including the multifunction syringe for 30 sec between patient [18]. In addition, a specific protocol using a disinfecting agent must be followed in the morning and at the end of the working week [19]. A number of efficient products are available on the market that can be applied onto dental units such as, hydrogen peroxide, hydrogen peroxide colloidal silver, hydrogen peroxide and silver, alkaline peroxide, active chlorine dioxide, chlorine dioxide, stabilized chlorine dioxide [20]. However, the inability to appropriately follow instructions and water-cleaning procedures itself is decisive in contamination of the DUWLs. According to a European survey on general dentistry practitioner's attitude on microbiological danger associated with DUWLs, the majority of the dentists did not clean, disinfect or assess the microbial load in the DUWLs [21]. Moreover, patient's fluids may play a crucial role in the bacterial growth inside the DUWLs. Indeed, saliva and other contaminants, such as blood and debris resulting from dental treatment, can be sucked back from the oral cavity into the tubules if anti retraction valves of the dental instruments are faulty or not working properly [22].

Contaminated aerosols can remain harmful and contagious for 24 h after a dental procedure [23] and their inhalation can lead to asthma, rhinitis, allergic alveolitis, and other dangerous diseases [24]. Dental aerosols also produce a cloud of microdroplets of a size $<1\mu$m, which remain suspended in the air and can potentially penetrate directly into the lungs [25]. Most recently, the Covid-19 pandemic has posed a serious setback in the

dental profession as the SARS-CoV-2 virus can spread in the aerosols generated during dental treatment and be responsible for cross infections in the dental office [26].

As contamination in the dental office has lately become the main subject of the research community including well-known dental chair companies, a new device allowing the ejection of sterile water and any disinfection agent as well as a flow of air free of all contaminants has been recently developed. This device can solve the contamination problem when doing some sensitive dental treatment such as implant surgery, bone and tissue grafting and bonding of resin composites especially in patients with health problems.

Considering the increased risk of contaminated DUWL to public health, the aim of this study is to evaluate and compare the levels of bacterial contamination in the output water between 2 types of DUWL, A-dec (A-dec™ Performer 200, Newberg, USA) and KaVo (ESTETICA™ E30/E70/E80 Vision, Kavo, Biberach, Germany). In the A-dec type, the disinfecting process is done manually whereas in the KaVo type it is automated. Indeed, an "inbuilt" bottle, which contains specific disinfectant agents is added to the main unit and is able to flush the entire DUWL system [27]. The null hypothesis tested was that there would be no difference in the bacterial contamination between the KaVo type and the A-dec conventional dental chair.

Materials and Methods

Study design and sample size

The study was conducted at a renowned dental polyclinic in Beirut, Lebanon. Three A-dec dental units along with 3 KaVo units were available in the clinic at the time of sampling. A total of 108 water samples were collected from the 6 chairs. The bacteriological analysis of the outlet water was carried out at the Laboratory of Pathogens (LAP) of Saint Joseph University of Beirut, Lebanon. The approval of the research ethical committee of the Saint-Joseph University of Beirut, Lebanon (USJ-2020-17) was granted.

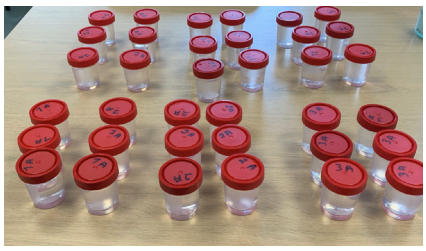

Figure 1. Water sample collection from the air-water syringe of the type DUWL in sterile bowls. Each sample taken in 6 replicates.

Dental chairs characteristics

All dental equipment in the clinics were in perfect working conditions with a periodic maintenance being done by professional technicians every 3 months. The mean age of the dental systems varies around 3 to 5 years old. All the dental chair units were connected directly to the same main municipal water reservoir source. A water safety filter BWT PURE AQUACALCIUM OSMOSISR (Mondsee, Upper Austria, Austria) was installed on the main water supply tank to provide the polyclinics with water of acceptable microbiological characteristics. Thanks to its reverse osmosis technology, the filter eliminates more than 95% of the water impurities (pesticides, nitrates, chlorine, bacteria, viruses). Bacteriological assessment of the filtered-water was conducted on a regular basis to make sure having water that meets the Lebanese Standards Institution (LIBNOR) norms for drinking water (i.e., ≤ 20 CFU/mL of heterotrophic water bacteria). Only the KaVo type dental chair is equipped with an automated sanitation system for bacterial eradication.

Water samples collection

Water samples were collected from the air-water syringe of each dental chair. According to the recommendations of the LAP technicians, each sample was taken in 6 replicates. Three samples were taken for each dental unit over a period of 3 consecutive days right after an ordinary working day, all under same conditions. It is to note that a flushing of 30 sec was made before samples collection.

Water samples were collected aseptically in 100 mL sterile plastic bowls (Heinz Herenz, Germany) as shown in Figure 1, and sent directly to the LAP for bacteriological analysis. Analyses of total aerobic flora count at 37 °C, and other microorganisms frequently associated with biofilms such as *P. aeruginosa*, faecal coliforms, total coliforms, faecal *streptococci* and sulfite-reducing anaerobic flora were performed (Table 1).

Operating protocol for microbiological analysis

Membrane filtration technique was used for water microbiological analysis as shown in Figure 2. This technique consists of a reduced pressure filtration system that requires a water pump, a filter holder with a base and a safety valve, filtration membranes, single-use plastic cups, clamp, and culture media.

All equipment was placed next to an electric nozzle to provide a sterile working area and to be able to sterilize the equipment. The base and the filter support were flamed for at least 20 sec each time before use. Once the support has cooled, the sterile filtration membrane was placed under sterile conditions. The sterile cup was then positioned on the base without damaging the membrane and the pump was connected to the electric current. A volume of 100 mL of water was gently poured into the cup. The valve of the filter support was then opened and the pump switched on by pressing the power button. Once the water was filtered, the tap was closed, the pump switched off and the membrane was then removed and incubated on specific medium.

Table 1. Bacteriological analysis carried out on all water samples.

	Total aerobic flora	*P. aeruginosa*	Faecal coliforms	Total coliforms	Faecal *Streptococci*	Sulfite-reducing anaerobic flora
Volume	100 mL	100 mL	100 mL	100 mL	100 mL	100 mL
Culture Medium	Plate count agar (PCA)	Cetrimide agar	mFC	MFendo	Slanetz	SPS
Growth Conditions	37° C for 24 h	37° C for 24 h	44°C for 24 h	37° C for 24 h	37°C for 48 h	44°C for 24 h
Interpretation	Total count	Fluorescent green colonies	Blue colonies	Rose to pink with metallic luster colonies	Pink or red colonies	Black colonies
Confirmation	NA	Gram stain Oxidase test API 20 NE	NA	NA	Black colonies on bile-esculin-azide agar (BEA) medium	NA

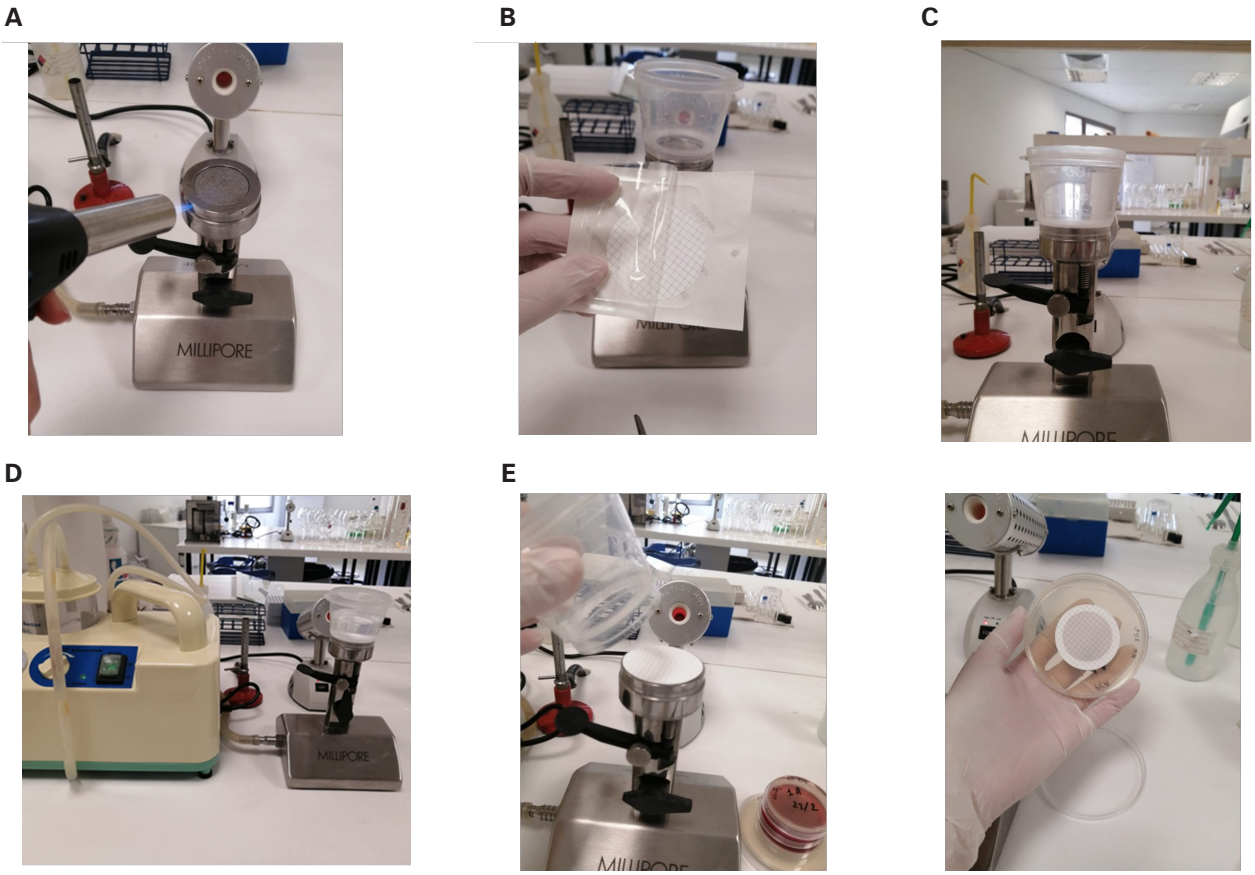

Figure 2. Membrane filtration technique used for water microbiological analysis
A. The support being flamed for at least 20 sec to ensure sterility; **B.** A membrane filter of 0.45μm to be placed on the support with grid side up using a sterile forceps; **C.** Filter funnel attached on the flask; **D.** Water sample being filtered through the membrane filter under gentle vacuum; **E.** The membrane filter being transferred to PCA culture medium.

Table 2. Colony count of various bacterial parameters found in 100 mL of each water sample collected from A-dec and KaVo DUWL presented as CFU/mL.

Water samples†	Total aerobic flora	*P. aeruginosa*	Faecal coliforms	Total coliforms	Faecal *Streptococci*	Sulfite-reducing anaerobic flora
A-dec 1	$>10^4$	$>10^2$	0	$>10^2$	0.1	0
A-dec 2	$>10^4$	$>10^2$	0	$>10^2$	0	0
A-dec 3	$>10^4$	$>10^2$	0	$>10^2$	0	0
A-dec 4	$>10^4$	$>10^2$	0	$>10^2$	0	0
A-dec 5	$>10^4$	$>10^2$	0	$>10^2$	0	0
A-dec 6	$>10^4$	$>10^2$	0	$>10^2$	0	0
A-dec 7	$>10^4$	$>10^2$	0	$>10^2$	0	0
A-dec 8	$>10^4$	$>10^2$	0	$>10^2$	0	0
A-dec 9	$>10^4$	$>10^2$	0	$>10^2$	0	0
KaVo 1	$>10^4$	$>10^2$	0	$>10^2$	0.02	0
KaVo 2	$>10^4$	$>10^2$	0	$>10^2$	0.05	0
KaVo 3	$>10^4$	$>10^2$	0	$>10^2$	0.1	0
KaVo 4	$>10^4$	$>10^2$	0	$>10^2$	0	0
KaVo 5	$>10^4$	$>10^2$	0	$>10^2$	0	0
KaVo 6	$>10^4$	$>10^2$	0	$>10^2$	0	0
KaVo 7	$>10^4$	$>10^2$	0	$>10^2$	0	0
KaVo 8	$>10^4$	$>10^2$	0	$>10^2$	0	0
KaVo 9	$>10^4$	$>10^2$	0	$>10^2$	0	0

†3 water samples from each dental unit over 3 consecutive days

Table 3. Percentages of A-dec and KaVo DUWL according to the number of bacteria presented as CFU/mL.

Bacterial parameter	CFU/mL	A-dec (n=9†)	KaVo (n=9†)	-*p*-value
Total aerobic flora	$>10^4$	9(100%)	9(100%)	-
Pseudomonas aeruginosa	$>10^2$	9(100%)	9(100%)	-
Faecal coliforms	0	9(100%)	9(100%)	-
Total coliforms	$>10^2$	9(100%)	9(100%)	-
Faecal *Streptococci*	0	8(88.9%)	6(66.7%)	0.718
	0.02	0(0.0%)	1(11.1%)	
	0.05	0(0.0%)	1(11.1%)	
	0.1	1(11.1%)	1(11.1%)	
Faecal *Streptococci* (dichotomized)	Presence	1(11.1%)	3(33.3%)	0.576
	Absence	8(88.9%)	6(66.7%)	
Sulfite-reducing anaerobic flora	0	9(100%)	9(100%)	-

†3 water samples from each dental unit over 3 consecutive days

Statistical analysis

A general linear model procedure of Statistical Package Software for Social Science (IBM SPSS, version 27.00, SPSS Institute Inc., Chicago, IL, USA) was used for the statistical analysis. The level of significance was set at -*p*-value ≤0.05. Percentages and frequencies were used to describe the amount of Colony-Forming Unit (CFU) for each bacterial parameter in the water collected from each chair type. Fisher's Exact test was used to compare the obtained percentages.

Results

The colony count of various bacterial parameters found in 100 mL of each water sample collected from A-dec and KaVo dental chairs is presented in Table 2. In Table 3, the prevalence of A-dec and KaVo chairs for each type of bacteria are shown as percentages.

As demonstrated in Table 2, the bacteriological analysis showed the presence of various bacteria in all water samples collected from both DUWL types with no significant statistical difference between the two types of dental chairs included in this study ($p > 0.05$).

Total aerobic flora at a level $>10^4$ CFU/mL is found in all collected samples from A-dec (100.0%) and KaVo (100.0%) dental chairs. Similar results are observed for *P. aeruginosa* and total coliforms. Indeed, levels $>10^2$ CFU/mL of *P. aeruginosa* and total coliforms are detected in all collected samples from the 2 DUWL included in this study (100.0% for A-dec and KaVo). As for faecal *Streptococci*, it is detected in 11.1% of A-dec chairs and 33.3% of KaVo chairs with no significant statistical difference between the two DUWL types ($p = 0.576$). Note that faecal *Streptococci* is detected at a level of 0.02 CFU/mL for one KaVo chair, 0.05 CFU/mL for another one KaVo chair, and 0.1 CFU/mL for one A-dec chair and one KaVo chair. On the other hand, faecal coliforms and the sulfite-reducing anaerobic flora were not found in any of the water samples collected from the two chairs.

Discussion

The quality of dental unit water is of significant importance since patients and dental staff are regularly exposed to water and aerosols engendered by the DUWL [28]. Recently, iatrogenic infections caused by the irrigation system of dental chairs have become the focus of researchers around the globe [29-30].

Water for testing was taken from the air/water syringes of two DUWL types, A-dec and KaVo. The obtained results revealed the presence, in both types, of various bacteria at high levels that are thousands of times greater than the limits of 100 CFU/mL set up by the ADA and accepted by the CDC [31-32]. According to the literature, contamination of DUWL has been previously described, and microbial levels of 10^4–10^6 CFU/mL have been reported in DUWL water samples. In accordance with data stated by other studies, the total bacterial counts in the collected water samples from the two DUWL included in this study were higher than 10^4 CFU/mL [33] indicating very poor water quality that is unpleasant for all patients. Our results show no statistically significant differences between the A-dec and KaVo DUWL type in terms of type and quantity of bacteria studied ($p > 0.05$). The null hypothesis stating that the KaVo type, which has an automated mode of decontamination, would result in no statistical difference in bacterial contamination than the A-dec conventional type is accepted. The origin of microorganisms in the DUWL is either the source of the water itself or the fluids coming from the oral cavity of the patients by an inverted suction mechanism.

Moreover, the output water of the two DUWL types ejected by the air/water syringe yielded *P. aeruginosa* at a density higher than 10^2 CFU/mL making theses dental units an infection source of *P. aeruginosa*. Similarly, a study conducted at a dental teaching center in Jordan, aiming to evaluate the extent of *P. aeruginosa* contamination in water samples from DUWL of the air/water syringe at three sampling times (at the beginning of working day, after 2 min of flushing, and at midday), showed counts ranging between 0 and 9.4×10^3 CFU/mL [10]. Moreover, a recent study reported *P. aeruginosa* contamination in 68% (13/19) of water samples from DUWL [34-35]. *P. aeruginosa* colonizes the respiratory epithelium of immunocompromised patients with chronic lung diseases including those with cystic fibrosis [36]. The vital prognosis of these patients would be engaged when antibiotic therapy proves to be insufficient [37]. In such patients, exposure to *P. aeruginosa* must be avoided at all times, as this microorganism is the main cause of lung destruction and premature death in these patients. In addition, the presence of Gram-negative bacteria in DUWLs can lead to the production of endotoxins (lipopolysaccharides) in the water and air of a dental surgery, which can induce inflammation and fever as an immune response in organisms [34]. Besides, *Pereira* in 2017 stated a relationship between *P. aeruginosa* and brain abscess [38].

On the other hand, our study revealed the massive presence of total coliforms at levels exceeding 10^2 CFU/ml. The results of studies conducted by Watanabe *et al.* [39] and Aprea *et al.* [40] were negative for bacteria of the coliform group. However, in our study, all samples were contaminated with total coliforms indicating that no tested water samples match the recommended quality of drinking water. Total coliforms are *Enterobacterales*, which include bacteria that are found in the soil, in water that has been influenced by surface water, and in human or animal waste. They are rod-shaped,

aerobic or facultative anaerobic bacteria possessing the enzyme ß-galactosidase, which releases a chromogenic agent used in culture media to identify them [41-43]. The main bacterial genera included in this group are: *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella* and *Serratia* [44-45]. However, their presence in water indicates that disease-causing organisms could be in the water system. The origin of such microorganisms in the DUWL come most probably from the source of the water itself or from the fluids coming from the oral cavity of the patients by an inverted suction mechanism [46].

A study conducted by Chua *et al.*, [47] showed the absence of both faecal coliforms and faecal *Streptococci*. In the present study, the obtained results did not reveal the presence of faecal coliforms in both type of dental units, nevertheless it showed the presence of faecal *Streptococci* in 11.1% of the water samples collected from the A-dec unit and 33.3% from the KaVo unit. Faecal coliforms are the group of the total coliforms that are considered to be present specifically in the intestine and feces of warm-blooded animals. Because their origins are more specific than the origins of the more general total coliform group of bacteria, faecal coliforms are designated as a more accurate indication of animal or human waste in water than the total coliforms. Like faecal coliforms, faecal *Streptococci* are not common causes to human infection [48]. Therefore, the faecal *Streptococci* analysis was performed to gauge whether or not tested water from the two DUWL types is frequently or potentially infected with bacteria. The obtained results demonstrated that 1/3 of the samples collected from the KaVo type are not of safe quality. Whereas, 1/9 of the samples collected from the A-dec type are considered as unpleasant for all patients. Faecal *Streptococci* are low-grade pathogens and rarely are linked to human disease. However, they may cause urinary tract infection in catheterized patients; mixed abdominal wound infections following gut surgery; and endocarditis on abnormal valves [47].

Sulfite-reducing anaerobic bacteria are generally considered as indicators of clostridial contamination in water, and are often associated with faecal contamination. Within 24 h, sulphite-reducing bacteria reduce sulphite to sulphide at 37° C. Among the various species of these bacteria, *Clostridium perfringens* is the most important organism. They are obligate anaerobic bacilli with unusual features: spore formation, toxin production [49]. In this study, no water samples collected from both DUWL types are contaminated with sulfite-reducing anaerobic bacteria.

Nowadays, despite the application of disinfectants, bacterial contamination seems to persist.

Indeed, in spite of being equipped with an automated disinfection system allowing the disinfectant flow in all water ducts of the dental chair, the water from the KaVo type seem not to have a better quality than that of a regular one. Moreover , the hoses of the instruments have to be plugged into the hygienic center of the chair during the disinfecting cycles. This has to be done in the morning, between patients and at the end of the working day. A specific program, which contains higher concentration of disinfectant (oxygenal, KaVo, Biberach, Germany) is done weekly.

In fact, our results did not show any significant statistical differences between the KaVo and the A-dec dental chair that is not appointed with a disinfection system. This could be explained by the dentist's work overload as well as the underestimation of the risk of infection that can be caused by water contamination in dental chairs, leading to a poor management of decontamination protocols and an abstention of the dental equipments' periodic maintenance [50]. As previously mentioned, if disinfection protocols are not monitored promptly, there will be formation of bacterial biofilm resistant to disinfectants.

Studies have shown that the quality of dental chair unit input and output water was significantly different [51-53]. The bacteriological analysis of the water at the source showed significantly lower bacterial cell density than of the output water. For instance, municipal water supplied to the units yielded an average aerobic heterotrophic bacterial cell density of 287 CFU/mL. However, the corresponding density in output water was considerably higher; the average cell density in water from the air/water syringe was 6440 CFU/mL [54]. Indeed, DUWL involves polystyrene-made tubing that carries water to the high-speed handpiece, air/water syringe, and ultrasonic scaler promoting bacterial growth and development of biofilm due to the presence of long narrow-bore tubing, inconsistent flow rates, and the potential for retraction of oral fluids. The latter will be responsible for continuous contamination of the irrigation network of dental chairs by releasing fragments containing aggregates of bacterial cells and therefore plays the role of a reservoir of opportunistic pathogens resistant to disinfecting agents [55]. It is of note that a low flow of water in these tubules during the operation of dental chairs, as well as the stagnation of water at the time of work stoppage constitute factors favoring bacterial multiplication and biofilm formation [56].

A study carried out by Dang *et al* in 2022 [57] showed a significant difference in the bacterial community according to the dental specialty whether it was endodontics, periodontology or prosthodontics but *Pseudomonas* and *Acinetobacte*r were dominant in the DUWL whatever the specialty was. These pathogenic bacteria for humans have also been detected in other studies [58-60]. *P. aeruginosa*

has the ability to multiply in an environment with low nutrient supply and shows resistance to disinfecting agents [61]. A study conducted by Cancan Fan in 2021 [62] also confirms that the nature of bacterial biofilm can be influenced by the dental specialty practiced on the dental unit. The age of the dental unit as well as the total number of patients play an important role in the general composition of the biofilm.

Moreover, Sina Dobaradaran *et al.* 2014 [54] demonstrated that there is a close relationship between the age of dental equipment as well as the number of working days and the bacterial density in the water network of the dental chair. Indeed, a comparison of the total number of bacteria between private and public chairs where the flow of patients is generally greater shows a higher bacterial density for municipal clinics compared to private clinics. This phenomenon can be explained by a failure of the anti-retraction valves which prevent the reverse aspiration of oral fluids inside the DUWL.

The presence of *P. aeruginosa* is the direct consequence of this failure. In this case, the bacteria are transported from the patient's mouth inside the tubules. Their small diameters as well as the stagnation of water promotes the adhesion of these bacteria as well as their multiplication. A study carried out by Jessica Lizzadro *et al.* in 2019 [63] evaluated the differences in microbiological contamination between two types of dental chairs: one type equipped with an anti-retraction valve and the other not. The result was a higher bacterial prevalence in dental units that are not equipped with an anti-retraction valve, in particular of *P. aeruginosa*. The latter is capable of inhibiting the growth of other bacteria by secreting molecules called bacteriocins [64-65]. Indeed, a study conducted by Xue-Yue Ji *et al.* in 2016 [66] to assess the effectiveness of anti-retraction valves in dental units showed a failure of these valves in 51.72% of the dental units evaluated. Some units showed reverse aspiration of oral fluid greater than 100 μL of oral fluid. This volume exceeds largely the safety threshold set by the ADA. Any aspirated volume greater than 40 μL indicates a failure of the anti-retraction valves resulting in contamination of the DUWL.

It is recommended that the medical staff carry out intensive decontamination as well as rinsing of all instruments between patients [67]. A study done by Hami in 2018 [68] evaluated the effect of rinsing on the reduction of bacterial flora, particularly *P. aeruginosa* and Legionella, in the irrigation system of dental units. The results showed a drop in bacterial counts after rinsing but the amount was still higher than normal levels set by the Environmental Water Protection Agency. The authors suggested an increase in rinsing time as well as an implementation of an advanced chemical disinfection protocol for dental units.

Some bacteria have an extremely high adhesion capacity and are therefore resistant to most decontaminating agents. This is particularly the case of *P. aeruginosa*, which represents the pathogenic bacterium par excellence for humans [69-70].

Fatima Abdouchakour *et al.*, 2015 [71] highlighted a high level of *P. aeruginosa* exceeding the acceptable threshold defined by the CDC. The risk for the patient increases especially in surgical interventions such as gingival grafting, implant placement, dental extractions. The latter interventions require also a water deprived of any contaminant in order to maximize the treatment success for the long term [72].

Medical personal is also at risk because inhaling aerosols containing this bacterium will cause respiratory infections, which can be harmful and even lethal for health compromised individuals.

Rachel M. Monteiro in 2018 [73] also implemented a flushing protocol to try to find a solution to bacterial colonization. The experiment consisted of completely emptying the water tank of the dental chair in the morning and evening with rinsing between patients without the use of chemicals. The result was a decrease in bacteria but it was still at an unacceptable rate.

The use of chemical disinfecting agents based on hydrogen peroxide, sodium hypochlorite, ethylene di-amino tetra acetic acid can reduce bacterial biofilm but they can compromise the integrity of the DUWL [74]. Consequently, the contact surface with the microorganisms increases and thus promotes biofilm's formation. The use of disinfecting agents may present certain limits related to the phenotypic changes of bacteria [75], the resistance of certain bacteria constituting the biofilm and finally the long-term toxic effect for the patient and medical staff [76].

Furthermore, they can have a negative effect on the bond strength of composite resins. Indeed, the water, mixed with the decontamination agent, is ejected by the air/water syringe of the dental unit for the rinsing of the ortho phosphoric acid. This could have consequences on the adhesion of composite resins given the lack of information in the literature on the possible interaction with the adhesive. Further studies are needed to assess the impact of decontaminating agents on the bonding of composite resins.

Humidity may also be increased in the air syringe leading to a lower bonding of resin composites [77].

As a possible solution to the bacterial contamination of the DUWL, other than implementing a strict disinfection protocol, DentiPure KM™ (KM, Beirut, Lebanon) could show some benefits in some dental application. Indeed, it is a new device that allows sterile water to be ejected independently of the dental units' supply circuits. It is designed in a way that the tubes, responsible for the water

and air flow, can be detached from the device at the end of the day in order to be able to sterilize them. This being impossible to achieve in conventional dental units where the only solution to permanently eradicate the bacterial biofilm is to change all the DUWL which will cost a fortune from an economic point of view but also a significant waste of time for the practitioner. This device could be attached to any brand of dental units whether new or old and will find its use in all dental specialties from simple restoration to advanced surgeries where the use of sterile water, free of any bacteria, are more than necessary for the success of the treatment and to avoid the development of an infection. It should be noted that this device has the possibility of ejecting any type of disinfectant, such as chlorhexidine, in addition to sterile water. The beneficial role of chlorhexidine is now well known, especially in the long-term adhesion of composite resins. The limitation of the study resides in the fact that a small number of water samples were used. Further analysis including a higher number of samples collected at various time of the day is to be done. Moreover, the quality of dental chair unit input and output water should be analyzed to determine the exact source of bacterial contamination. It should also be noted that the collection of the specimens for culture is a very critical step that should be done properly in order not to have false results.

Addressing these limitations and exploring future perspective will contribute to a better knowledge of the bacterial contamination of the DUWL.

Conclusions

Despite all the solutions considered to try to eradicate the bacterial biofilm formation, the problem remains one of the greatest challenges in modern dentistry. Practitioners and medical staff should not underestimate the harmful consequences of this bacterial growth not only on the health of their patients but also on their own health. Strict application of hygiene protocols by medical personnel is necessary to minimize bacterial proliferation. Moreover, future dental chair design must attempt to resolve the problems associated with microbial contamination of the water supply and aerosols generated during dental procedures.

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