Scientific Research Publishing

Advances in Infectious Diseases, 2024, 14, 857-872
https://www.scirp.org/journal/aid
ISSN Online: 2164-2656
ISSN Print: 2164-2648

Cross Contamination in Oral Health Care: A Potential Public Health Risk

—The Phenomenon of Biological Fluid Backflow: An Experimental Study

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How to cite this paper: Alaoui, H.H., Gharibi, A., Timinouni, M. and Zaim, N. (2024) Cross Contamination in Oral Health Care: A Potential Public Health Risk. *Advances in Infectious Diseases*, **14**, 857-872.
https://doi.org/10.4236/aid.2024.144064

Received: November 16, 2024
Accepted: December 22, 2024
Published: December 25, 2024

Abstract

In oral health care, the spread of harmful infectious agents from the oral cavity is a constant concern. The aim of this study was to evaluate the possibility of cross-contamination between patients due to the backflow of biological fluids and contaminated aerosols into the water tubes of rotating instruments (high-speed turbines). A second aim was to assess the dispersion of the aerosols generated in the medical environment and the risk of contamination for the dentist. Materials and methods: For safety reasons, we carried out an experimental study on two sheep heads purchased from a butcher to simulate periodontal treatment in the two oral cavities. The first cavity was artificially contaminated with the reference strain of *Staphylococcus aureus* to assess the transfer of this bacteria from one cavity to the other through the waterlines of the high-speed turbine used. Results: The study revealed a worrying risk of cross-contamination from contaminated aerosols flowing back through the dental turbine into the dental unit waterlines (33.33%) [$p < 0.05$]. During dental procedures, aerosols and water droplets containing *S. aureus* and total heterotrophic bacteria (THB) were dispersed at varying distances from the oral cavity. In particular, the highest contamination levels were found within 0.3 m of the patient for *S. aureus* ($M = 43.66$, $SD = 1$) and THB ($M = 45.59$, $SD = 5$), with contamination levels decreasing at a distance of 1.5 m, respectively ($M = 5.63$, $SD = 3.61$; $M = 8.09$, $SD = 7.01$) [$p \leq 0.05$]. Conclusions: Procedures should be implemented to limit the risk of cross-contamination during dental treatment. This can be achieved by strict adherence to hygiene and asepsis measures in the dental unit and medical devices, compliance with regulatory standards (e.g., ISO 15883 1-2), and the installation of anti-retraction valves in dental turbines, dental chair

unit and suction systems to prevent backflow of contaminated biological fluids and aerosols.

Keywords

Cross Contamination, Aerosols, Dental Unit Waterline(s), Back-Contamination

1. Introduction

In dentistry, we cannot talk about healthcare-associated infections in the strict sense of the word, as current practice does not involve hospitalisation. However, during dental treatment, we encounter various infectious agents, including bacteria such as *Streptococcus pneumoniae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Legionella pneumophila* and *Pseudomonas aeruginosa*, and viruses such as hepatitis B virus, hepatitis C virus, human immunodeficiency virus, herpes simplex virus and, more recently, the SARS-CoV-2 virus. These micro-organisms can be transmitted by direct contact with bodily fluids such as blood, saliva, pus and respiratory secretions, or by specific environmental components of the dental practice (e.g., aerosols generated during treatment, tap water), or by indirect contact via contaminated surfaces, medical devices or the soiled hands of the dentist or assistant. This has potentially serious public health implications. Dental care remains a risk factor for infection, particularly in immunocompromised patients [1]-[3].

Regular use of air- or water-cooled rotary or ultrasonic instruments promotes the generation of aerosols contaminated with viruses and bacteria present in the patient's oral cavity and upper respiratory tract. These micro-organisms are dispersed outside the patient's oral cavity and are usually adsorbed onto larger particles, which serve as both a growth medium and a transmission vector [3]-[6]. A recent study by van Doremalen *et al.* showed that SARS-CoV-2 has a survival time of three hours in aerosols and up to three days on solid surfaces such as metal or plastic [2].

Furthermore, an influential study by Kobza *et al.* (2018) delved into the impact of bioaerosols on the contamination of dental professionals. The results revealed a significant range of bacterial sprays, ranging from 1.86×10^5 to 4.3×10^5 bacteria/m^3, depending on the specific procedure performed. In particular, oral cavity preparation yielded bacterial sprays ranging from 24 to 105 CFU/m^3, ultrasonic scaling from 42 to 71 CFU/m^3, and oral examination from 24 to 62 CFU/m^3. These findings highlight the risk of cross-contamination, both from patient to practitioner and from patient to medical environment [7]. The occurrence of cross-contamination in oral-dental care has tragically resulted in fatal cases, particularly in immunocompromised patients and dental staff. These incidents are associated with exposure to contaminated aerosols and water droplets during procedures,

often involving high-speed dental turbines, air/water syringes, and sonic and ultrasonic scalers. It is vital that these risks are addressed to prevent further harm. The first case of a dentist dying as a result of cross-contamination in dental surgery occurred in 1995; the opportunistic pathogens *Legionella pneumophila* and *Legionella longbeachae* were detected in the dentist's lung tissue and in the dental unit waterlines (DUWLs) used during treatment [8] [9].

Two cases of fatal *Legionella pneumophila* contamination have occurred in dental settings. In Sweden, an immunocompromised patient died after exposure to the bacteria during dental treatment [10]. In Italy, an 82-year-old patient died after inhaling water droplets contaminated with Legionella pneumophila during a routine dental examination [11]. A German study (2023) found that there is a recurrent risk of infection for both medical staff and patients due to cross-contamination by Legionella in dental settings. This risk is attributed to the aerosolization of water used during dental procedures, and factors such as the equipment used, the medical environment, disinfection methods and temperature play a role in this cross-contamination [12].

Dental cross-contamination is now recognised as a major public health problem worldwide. In Morocco, awareness of this risk and its environmental impact is still limited. The aim of this study was to assess the potential for biological fluids and contaminated aerosols to re-enter DUWLs, resulting in cross-contamination. It considered both patient-to-patient and patient-to-medical environment cross-contamination during routine dental procedures.

2. Materials and Methods

The transmission of *Staphylococcus aureus* (*S. aureus*) from one patient to another was simulated using a water-cooled rotary instrument commonly used in dental care. For safety and ethical reasons, an animal model, *Ovis aries*, was used as its oral cavity structure is similar to that of humans. The sheep heads (*Ovis aries*) used were commercially available (from butchers) and intended for human consumption. The aim of this experimental study was to demonstrate the risk of cross-contamination during dental treatment due to the physical phenomenon of backflow of biological fluids at the outlet of waterlines associated with rotating instruments (high-speed turbines).

2.1. Study Site

The study was conducted in 2023 in a closed room with a single dental unit (individual perio-dental surgery room) in the periodontology department. The study lasted 2 hours, including 30 minutes for the preparation of the sheep's oral cavity, 40 minutes for the simulated dental treatment, 20 minutes for the sterilisation of the dental turbine and 30 minutes for the swabbing of the surfaces before and after the hygienist's intervention. Before starting the study, we obtained the consent of the Director of the University Dental Centre and the approval of the Research Committee of the Dental Consultation and Treatment Centre.

2.2. Selection of Bacterial Species for Artificial Contamination

S. aureus is a gram-positive bacterium that is both a human commensal and a pathogenic opportunistic agent responsible for serious nosocomial and community-acquired infections [13].

Research has shown that the oral cavity can serve as a source of *S. aureus*, leading to transmission to other parts of the body and even to the environment. The importance of the oral cavity as a site of *S. aureus* colonisation and transmission has been demonstrated in studies [13] [14]. In addition, the emergence of methicillin-resistant *S. aureus* (MRSA) strains has made the treatment of *S. aureus* infections challenging. There is also evidence that penicillin-resistant staphylococci are prevalent in subgingival biofilm, regardless of the periodontal status of the patient [15]. Considering these factors, we chose methicillin-susceptible *Staphylococcus aureus* (MSSA) as the bacterial species for our experimental study. Our aim is to investigate how this bacterium can spread and potentially serve as a source of broad-spectrum cross-contamination from a contaminated oral cavity.

2.3. *Ovis Aries* Oral Cavity Preparation and Disinfection

For this study, two medium-sized Ovis aries sheep heads [16] were prepared by rinsing, drying and disinfecting with a 10% betadine solution [Antiseptic Mouthwash - Laprophan, Casablanca, Morocco]. After leaving the betadine in contact for 3 to 5 minutes, the heads were thoroughly rinsed with sterile distilled water, followed by sodium thiosulphate (3.5 g/L) to neutralise and remove any residual betadine. Microbiological examination of both oral cavities was then performed by direct swabbing [sterile swabs—Humeau, Couëron, France] of the hard and soft palates, teeth and gums. The samples were inoculated on plate count agar [Plate Count Agar (PCA), Biolife, Milan, Italy] and incubated for 24 hours at 37˚C under aerobic conditions to confirm the efficacy of oral cavity disinfection at baseline (T0).

2.4. Artificial Contamination of the First Oral Cavity (1st Patient)

The reference bacterial strain used in our study was obtained from the Molecular Bacteriology Laboratory of the Pasteur Institute (Morocco). The oral cavity of the first patient was artificially contaminated under aseptic conditions with 5 ml of a bacterial suspension of methicillin-susceptible *S. aureus* with an optical density (OD) of 0.08 at 600 nm. The procedure involved dipping a sterile swab [Humeau in Courron, France] into the bacterial suspension and then inoculating the hard and soft palate, gums and teeth. Strict standard safety protocols were followed throughout the procedure to maintain aseptic conditions.

2.5. Dental Treatment and Cross Contamination Detection

The dentist was asked to perform dental treatment on the first oral cavity, simulating the conditions of dental treatment for routine scaling. A sterile high-speed dental turbine with a water-cooling system was used [model: NSK Ti-Max Z95L].

After performing the dental treatment on the first patient (oral cavity of the first animal), the high-speed dental turbine was thoroughly sterilised before being used on the next patient (oral cavity of the second animal). The dental turbine was taken to the disinfection and sterilisation department where it was cleaned with Wip'Anios Excel disinfection wipes (Laboratoires Anios™, Lezennes, France). A lubricant spray (Lubri-Fluid, Dental Bio-Air, Switzerland) was then applied to the inside of the handpieces. The dental turbine was placed in a sealed bag and autoclaved at 134˚C for 20 minutes at atmospheric pressure.

To highlight the possible phenomenon of biological fluids being pumped back into the internal lumen of the DUWLs when the high-speed dental turbine is continuously and abruptly stopped, which could be a potential source of cross-contamination. Samples were collected from the oral cavity (hard and soft palate, gums and teeth) with sterile swabs moistened with sterile distilled water at the end of the dental treatment of the second patient, who was assumed to be sterile. The swabs were identified (sample type, replicate, number and date) and sent to the microbiology laboratory for inoculation in triplicate on a selective medium (mannitol salt agar) [Biokar Diagnostics, Pantin Cedex, France]. After incubation for 48 hours under aerobic conditions at 37˚C, identification was made based on colony morphology, gram staining, standard biochemical tests and susceptibility profile to the usual antibiotics, *i.e.*, erythromycin (E-15UI), tetracycline (TE-30 µg), oxacillin (OXA-5 mg), vancomycin (VA-30 mg), rifampicin (RIF-5 µg), amikacin (AN-30UI) and ciprofloxacin (CIP-5 mg).

According to Clinical and Laboratory Standards Institute (CLSI) guidelines, bacterial inocula with 0.5 McFarland adjusted turbidity were plated on Muller-Hinton agar plates [Biokar Diagnostics, France] and antibiotic discs were aseptically applied [disc diffusion method]. After aerobic incubation at 37˚C for 18-24 hours, inhibition zones were measured and susceptibility was assigned. Susceptibility to methicillin in *S. aureus* was assessed using a cefoxitin disc (30 µg) as part of the standard susceptibility testing. Strains with an inhibition diameter greater than 22 mm were considered to be MSSA [17].

2.6. Assessing Airborne Contamination in the Operating Room

Before the start of the treatment (T0), the operating room was thoroughly cleaned and disinfected. Sedimentation dishes, 9 cm in diameter, containing a selective medium (MSA) and a non-selective medium (PCA) were placed in triplicate at different distances from the DCU: 0.30 m, 0.6 m, 1 m and 1.5 m for 40 minutes. Windows and doors were kept closed throughout the study to prevent external contamination.

Diffusion-controlled sampling allowed passive monitoring of the air in the operating room during the dental procedure (T1). For the 40 minutes of the patient's dental treatment, the plates were positioned at the same intervals: 0.3, 0.6, 1 and 1.5 metres. Again, doors and windows were kept closed to prevent contamination from the outside. After 48 hours of aerobic incubation at 37˚C, the results of the

plates were expressed as CFU (colony forming units) per plate every 40 minutes.

2.7. Evaluate Surface and Medical Device Contamination

Direct swabs were taken at three different times to assess the microbiological quality of surfaces and medical devices: T0 before dental treatment, T1 immediately after dental treatment and T2 after biocleaning of surfaces and medical devices (after hygienist intervention). Swabbing is the most appropriate surface sampling method for rough, sloping, inaccessible or wet surfaces, such as the surfaces of medical devices, according to ISO/DIS 14698-1 [18].

The sample consisted of 96 swabs (in duplicate) from the following surfaces and medical devices: dental unit suction hoses, micromotor handpiece, dental spittoon, dental chair, dental operator stool, dental operating lights, dental mirror, dental probe, dental air/water syringe, dental handpiece holder, floor surface, other surfaces, scrubs, gloves, surgical mask, goggles, medical gown and dental head caps. After moistening the swabs with sterile distilled water, samples were taken by squaring the area of the identified surface (1:1:1:1); in the case of syphons, the swab was dipped into the syphon with circular movements. The swabs were identified (sample type, replicate, number and date) and transported in a cool box (+4˚C) to the microbiology laboratory. Each swab was then placed in 5 ml of heart-brain broth [Biokar Diagnostics, Pantin Cedex, France] and subcultured on Mannitol Salt Agar (MSA) [Biokar Diagnostics, Pantin Cedex, France] and PCA plates (in triplicate), followed by aerobic incubation at 37˚C for 48 hours.

S. aureus was identified on the basis of colony morphology, gram staining, standard biochemical tests, and its methicillin susceptibility profile, as described above. Results are expressed in CFU/cm^2.

2.8. Statistical Analysis

The data were classified using Microsoft Office Excel and analysed using SPSS software (version 26.0, SPSS Inc., Chicago, USA). The Chi-square test was used to determine the significance of the differences observed at the different sampling times [T0, T1 and T2]. Descriptive statistics were used, reporting means (M) and standard deviations (SD) for quantitative data. All assumptions of the statistical methods were met and data were considered statistically significant if $p \leq 0.05$ at the 95% confidence level.

3. Results

3.1. Detection of Cross Contamination during Oral Care Procedures

After 48 hours of incubation at 37˚C, plates of MSA medium inoculated with a swab from the oral cavity of the second patient (considered sterile) showed bacterial growth in colonies with both macroscopic (yellowish colonies accompanied by fermentation of mannitol in the medium) and microscopic (gram-positive cocci arranged in diplococci and clusters) characteristics specific to *S. aureus*.

The antibiogram showed complete susceptibility to the antibiotics tested (me-

thicillin, erythromycin, tetracycline, oxacillin, vancomycin, rifampicin, amikacin and ciprofloxacin). These results indicated that the *S. aureus* species initially inoculated into the oral cavity of the first patient was likely transmitted to the subsequent patient, probably via the DUWLs or the dental instrument used.

The antibiogram showed complete susceptibility to the antibiotics tested (methicillin, erythromycin, tetracycline, oxacillin, vancomycin, rifampicin, amikacin and ciprofloxacin). These results suggest that the *S. aureus* species initially inoculated into the oral cavity of the first patient was likely transmitted to the next patient. The level of *S. aureus* contamination was 28.07% for hard and soft palate (M = 16; SD = 7), 33.92% for teeth (M = 19.33; SD = 7.77), and 38.05% for gingiva (M = 21.67; SD = 11.58) [$p \leq 0.05$]. This contamination is probably attributable to the DUWL or the dental instrument used. It should be noted that after the treatment of the first patient, the dental turbine used was sterilised before the treatment of the next patient.

3.2. Detection of Contamination of the Medical Environment during Oral Care Practice

During oral care, aerosols and water droplets carrying *S. aureus* were transported at different distances around the patient. At a distance of 0.3 metres from the source, the mean number of *S. aureus* CFUs found was 31 (M = 43.66, SD = 1); at 0.6 metres, the mean number of CFUs found was 17 (M = 23.94, SD = 3). At 1 metre, the mean number of *S. aureus* CFUs found was 19 (M = 26.76, SD = 3.61), and at 1.5 metres, the mean number of CFUs found was 4 (M = 5.63, SD = 3.61), indicating a statistically significant decrease in bacterial spread with increasing distance (43.66 vs. 5.63; $p \leq 0.001$).

These results indicate possible aerobic contamination of the medical environment around patients during dental treatment, with statistically significant differences between the two sampling times T0 (before dental treatment) and T1 (after dental treatment) ($p \leq 0.001$) (**Figure 1**).

Regarding the microbial contamination of the air by total heterotrophic bacteria (THB), the PCA showed significant aerobic contamination during oral care (T1). At a distance of 0.3 m from the source, the mean number of THB CFU found was 62 (M = 45.59, SD = 5), at a distance of 0.6 m the mean number of CFU found was 33 (M = 24.09, SD = 6). At a distance of 1 m, the mean number of THB CFU found was 30 CFU (M = 22.06, SD = 6) and at 1.5 m, the mean number of CFU found was 11 CFU (M = 8.09, SD = 7.01), indicating a statistically significant reduction in bacterial spread with increasing distance (45.59 vs. 8.09; $p \leq 0.001$).

This contamination is attributed to the continuous production of aerosols and water droplets carrying THB, which is unavoidable in dental practice. Samples taken from the medical devices and the surfaces showed a high microbial accumulation at T1 during dental care activities compared to T0 (0 CFU/cm^2) and at T2 (0 CFU/cm^2) immediately after biocleaning (**Figure 2**).

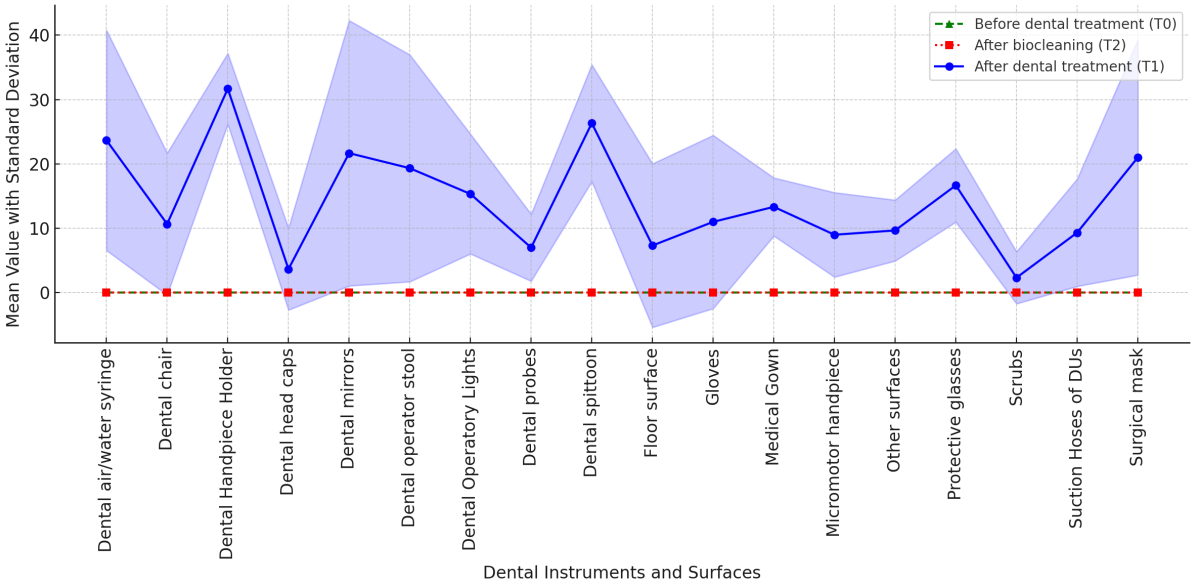

Figure 1. Medical environment and medical devices contaminated with *S. aureus* (CFU/cm²).

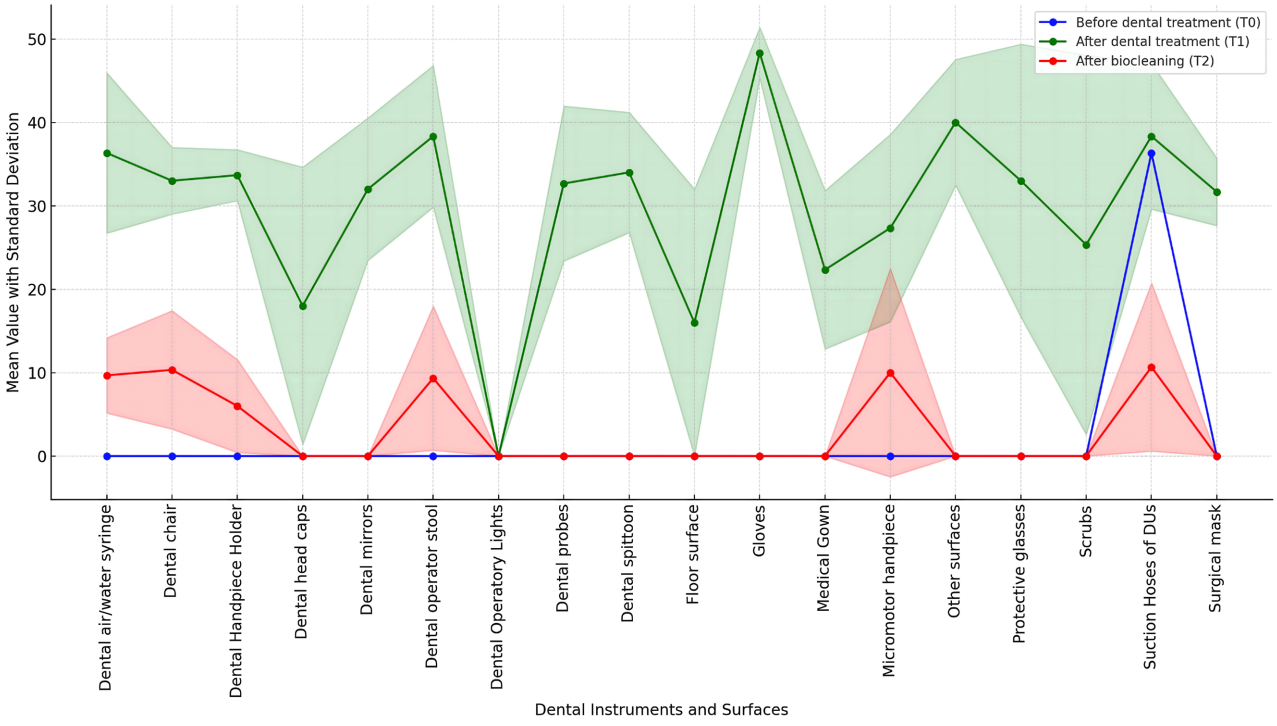

Figure 2. Medical environment and medical devices contaminated with THB (CFU/cm²).

4. Discussion

Microbiological contamination of dental care units (DCUs) is a significant oral health issue. DCUs are instruments used by dentists to provide dental care to patients. However, due to their frequent use and the presence of biological fluids, DCUs can become potential sources of microbiological contamination [12]. Our study builds on previous research on cross-contamination in the oral healthcare

setting, demonstrating back-contamination by transfer of *S. aureus* from the oral cavity of the first patient to the second through dynamic dental equipment (33.33%) [$p < 0.05$]. This phenomenon is typically caused by the passage of the bacterial strain through DUWLs, which are complex networks of fine polyurethane tubing that facilitate the formation of microbial biofilms [5] [6]. The continuous and abrupt stops of the high-speed dental turbine in the patient's mouth create a physical backflow phenomenon that causes the return of biological fluids (blood, saliva, pus, etc.) and contaminated aerosols into the internal lumen of the DUWLs. This is a major source of cross-contamination, posing a health risk to both patients and medical staff. Various experimental studies have demonstrated the backflow of biological fluids into the internal lumen of the tubing after the passage of a coloured solution in water circuits, with backflow occurring after 25 seconds of continuous operation after several stops [19] [20].

In oral care, water is used to cool instruments, rinse the mouth and remove debris, resulting in the release of micro-organisms into the air in the form of small aerosols (5 μm) and larger contaminated water droplets (>200 μm). These micro-organisms can be drawn into the narrow tubes of rotating instruments, where they can form biofilms in DUWLs. When water flow stops in DCUs, air and oral fluids contaminated with microbes are re-absorbed, creating biofilms in the waterlines.

When the water flow is reactivated, these biofilms can release particles that contaminate the oral cavity of subsequent patients [12] [21]. A recent study by Van der Weijden has clearly demonstrated that high-speed handpieces, air-water syringes and mechanical scalers are all aerosol-generating procedures that pose significant health risks to both patients and dental staff [22]. In addition, a separate study has highlighted the issue of back-contamination, with opportunistic pathogens such as *S. aureus* and *Pseudomonas aeruginosa* potentially contaminating dental equipment [23]. These findings highlight the critical importance of implementing robust infection control measures in dental settings.

Furthermore, this problem is generally a result of contamination in the water flowing from the DCU. Some authors [9] [12] [22] have suggested that biofilms formed in the DUWLs, together with overnight stagnation of water and, in many cases, the absence of adequate filtration and disinfection systems, contribute to this problem. This also supports the idea that when water and air emissions from syringes are frequently stopped, a brief depression is created which sucks in aerosols and external contaminant particles [1].

During oral care, water droplets loaded with *S. aureus* and THB were transported at different distances around the source (the patient's oral cavity), ranging from a distance of 0.3 m from the patient's mouth to a radius of 1.5 m, demonstrating the contamination of the air in the operating room where the study was conducted.

Our findings are consistent with the 1994 study by Bentley *et al.* and the 2018 study by Offner and Musset, which estimated that contaminated aerosols from the patient's oral cavity could disperse up to 1.5 m [24] [25]. It has also been shown

that dental aerosols generated during oral care directly contaminate surfaces around the patient over an area of 1.5 m^2 [26]. Mirhoseini *et al.* (2021) found that the mean concentrations of airborne bacteria were 52 - 1030 and 8 - 844 CFU/m^3 at distances of 0.5 and 2 m, respectively. Gram-positive bacteria were the most common in all samples, increasing the risk of cross-infection. The age of the DCUs, the total number of patients treated per day and the type of dental procedures performed influence the microbial aerosol concentrations in dental service air, particularly in environments with many patients, dental staff and dental students, such as dental schools [27].

Based on our findings, the combined use of goggles, surgical masks, sealed hoods and face shields effectively prevents contamination of the practitioner's facial and nasal areas. Our study showed that after almost 40 minutes of high-speed handpiece activity, the use of personal protective equipment (PPE) helped to limit bacterial contamination from aerosols and protect the dentist. The mean accumulation of *S. aureus* and THB varied according to the type of PPE used: dental caps (3.67 - 18), gloves (11 - 48.33), gown (13.33 - 22.33), goggles (16.67 - 33.0), scrubs (2.33 - 25.33) and surgical mask (21 - 31.67).

Our findings are consistent with a systematic review by Ghoneim *et al.* [20]-[24], which evaluated the types and effectiveness of personal protective equipment in reducing the risk of infection transmission between dentists and their patients during aerosol-generating procedures. The use of a combination of personal protective equipment is an effective strategy to minimise aerosol contact and prevent the spread of bacterial and viral contamination [4].

Our research has shown that *S. aureus* found in a patient's mouth can spread as contaminated aerosols and water droplets, resulting in significant contamination within a 0.3 m radius (43.66%) around the patient and the immediate environment ($p < 0.05$). In addition, we observed cross-contamination between the patient and the medical environment, with *S. aureus* present in almost all areas of the operating room where the study was conducted (**Figure 1**). This suggests the potential for cross-contamination during dental procedures. It is important to note that *S. aureus*, including methicillin-resistant strains (MRSA), can survive on dry surfaces for 7 days to 7 months [28]. Therefore, biocleaning of medical devices remains a critical step to limit the risk of cross-contamination and break the chain of microbial transmission. Our results showed a complete eradication of *S. aureus* and a remarkable decrease in THB after the hygienist's intervention at T2. Traces of THB contamination were detected in the dental chair (M: 10.33; SD: 7.09), dental handpiece holder (M: 6.00; SD: 5.57), dental operator's stool (M: 9.33; SD: 8.62), micromotor handpiece (M: 10; SD: 12.49) and DCU suction hoses (M: 10.67; SD: 10.07). A multicentre study showed that microbial contamination of the dental practice environment (surfaces, air and water) increased during the day. The authors suggest that this contamination is probably due to inadequate disinfection between patients and the lack of appropriate aspiration systems [1].

The aspiration of biological fluids allows the removal of saliva, blood and pus

from cellular debris, which promotes the development of a microbial biofilm within the tubes of the aspiration system. In addition, when the patient closes his or her mouth on the cannula, the contents of the suction hose flow back into the patient's mouth, posing a real risk of cross-contamination. A recent study demonstrated the role of suction hoses in microbial contamination in immunocompromised patients treated in dental clinics with the opportunistic pathogen *Pseudomonas aeruginosa*, which was found to be resistant to commonly used disinfectants [29]. These results highlight the importance of effective biocleaning after each clinical procedure, using appropriate products (concentration, shelf life, instructions for use, protection during use) and approved by clinical hygiene standards. According to the study by Djeribi and Zaghez, decontamination based solely on disinfectant diffusion remains inadequate and should prompt consideration of the need for regular cleaning of the inside of tubes and porous cavities to reduce the bacterial load of patient secretions [16]. Studies have highlighted the importance of external and internal biocleaning of rotating equipment tubing prior to steam sterilisation to ensure effective sterilisation [30] [31]. However, the internal cleaning of rotating devices remains a challenge due to the complexity of their structure and the small dimensions of the tubing. Nevertheless, Offner and Musset developed a method to validate the internal biocleaning of rotating devices according to the ISO 15883 standard for instrument biocleaning [30] [31]. In addition, Manli Liu *et al.* confirmed the effectiveness of the pulsed vacuum steriliser in improving the cleaning quality of dental handpieces and reducing the cleaning time. According to the authors, this sterilisation method should be widely adopted and promoted in clinical practice [32].

A simulation study of dental scaling and drilling on a dental mannequin, carried out in a single-patient examination room, showed that the individual use of a high-volume evacuator (HVE) or a commercially available air purifier reduced the aerosol accumulated during a 15-minute drilling procedure to a reduction rate of 94.8% to 97.6%. The simultaneous use of both measures increased the reduction rate to 99.6% [33].

In addition, Villa and Grenon [34] invented "The Cupola", a shield that creates a mechanical barrier around the patient's head and body. The Cupola was developed for providers working in the oropharyngeal region (dentists) and provides an additional layer of protection to the recommended personal protective equipment. The results of the pilot study showed a significant reduction in aerosols generated during simulated dental procedures when the Cupola was used. The mean number of 0.5 µm airborne particles was 65 ± 7 without the Cupola and 29 ± 28 with the Cupola. Similarly, the use of the Individual Biosafety Capsule in Dentistry (IBCD) proved to be an effective means of reducing the spread of bacterial and viral contamination by approximately 99% and 96%, respectively, around the main aerosol source ($p < 0.05$) [35].

In addition, innovative studies have introduced new strategies to minimise bacterial contamination and manage biofilm formation, including the use of chlo-

rogenic acid to treat DUWLs [36], and the use of plasma to sterilise DUWLs. Plasma sterilisation, which is part of electrochemically activated water, effectively reduces bacterial contamination and biofilms in DUWLs [37]. On the other hand, solid-phase nanometric silver (NMS) has shown bactericidal activity against bacterial biofilms formed in DUWLs, demonstrating the important role of solid-phase NMS in preventing contamination of DUWLs [38]. Surface disinfection using pulsed xenon ultraviolet technology is also an effective complement to established manual cleaning protocols and guidelines to significantly reduce contamination of frequently touched surfaces in the hospital environment [39].

An environmental method has been developed to eliminate pathogenic microorganisms from large areas and surfaces. This technology is based on the release of reactive oxygen species (ROS) in the form of hydroxyl radicals (OH-) and can be widely used for air and surface disinfection in dental clinics [40].

5. Conclusion

Dental care poses a significant risk of infection, particularly for patients with compromised immune systems. Our research highlights the issue of cross-contamination in oral care. Our findings clearly show that instruments such as high-speed dental turbines and air/water syringes connected to water cooling systems are common sources of transmission of infectious agents beyond the patient's oral cavity. Cross-contamination occurs primarily through the generation of aerosols and water droplets containing contaminated biological fluids and tissue debris, or through the backflow of these fluids via DUWLs, posing a threat to the health of both patients and dental professionals. To address this, it is critical to maintain strict hygiene and asepsis protocols, follow cleaning standards such as ISO 15883, and implement standard precautions for invasive procedures involving potential biological fluid spillage. These precautions should be universally applied and reinforced for high-risk patients.

6. Recommendations for Safer Dental Care

In order to reduce and prevent the risk of cross-contamination, significant efforts must be made in dental treatment centres. Our study has led to the following recommendations.

- Effective cleaning and disinfection methods should be used for flat surfaces, inclined surfaces, and cavities in DCUs. Swabs can be used to clean canals and hard-to-reach areas.
- A protocol for internal disinfection of DUWLs should be established after each clinical procedure to eliminate all traces of opportunistic pathogens and prevent biofilm formation.
- Install anti-retraction valves on rotating dental instruments (high-speed turbines), DCUs and suction systems to prevent backflow of biological fluids and contaminated aerosols.
- Regularly evaluate the effectiveness of anti-retraction valves and replace them

if they fail.

- Regular cleaning and disinfection of suction system filters, as well as the replacement of disposable filters, are essential to breaking the cycle of infection.
- Installing "ventilation decontamination systems" with high efficiency particulate air (HEPA) filters is recommended to reduce air contamination in departments.
- Incorporate a local exhaust ventilation system into the operational configuration to capture aerosols at source and limit their dispersal.
- Ongoing and up-to-date training of hygiene staff is essential to prevent cross-contamination during healthcare procedures.
- Further research into new technologies and innovations is required to limit the spread of aerosols and to reinvent the traditional configuration of DCUs and dental centres.

7. Study Limitations

Our study had five limitations:

1) The duration of the study (40 minutes) was relatively short, and does not allow an assessment of the long-term effects of cross-contamination, particularly with regard to the accumulation of biofilms, or the persistence of pathogens. Longer studies with more patients are needed.

2) The study was conducted in a single dental unit, which may not reflect the variability of cross-contamination risks in other environments or with different equipment. The dental services provided were limited to scaling and polishing.

3) The study focused on a single type of dental instrument (high-speed dental turbine and air/water syringe), which does not allow an assessment of the impact of other instruments or devices used in dentistry on cross-contamination.

4) The dental procedures performed on the two patients were homogeneous (scaling and polishing), making it impossible to assess the effect of other dental procedures on cross-contamination.

5) Environmental factors such as humidity and temperature in the treatment room were not controlled, which could have influenced the results of the study.

The advancement of oral health care requires comprehensive research in different specialties, such as orthodontics, dentofacial orthopaedics, prosthodontics, oral and maxillofacial surgery, endodontics, surgical odontology and paediatric odontology, as well as consideration of larger dental treatment areas. There is limited direct evidence on the risk of cross-contamination in oral healthcare settings. Therefore, further research is essential to accurately assess and estimate the risk of cross-contamination among different oral healthcare providers, including both public and private practitioners.

Conflicts of Interest

The authors declare no conflicts of interest.

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