Research on *staphylococcus spp* in biofilm formation in water pipes and sensibility to antibiotics

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Abstract
Water from dental equipment presents risks for surgeon-dentists as well as for patients because it might work as a means of dissemination/ transmission of microorganisms. The objective of this study was to verify the quality of the water used in dental equipment by means of microbiological analysis, accomplishing the count of *Staphylococcus spp*. There have been collected 160 samples of water from reservoirs, taps used for hand washing, air-water syringes, and high-speed handpieces, in 40 dental offices in the city of Barretos, São Paulo. The rules concerning bacteriological analysis in cfu/mL from Standard Methods for the Examination of Water and Wastewater have been followed. The analysis of the results has made it possible to verify that out of the total of samples, 28% did not meet the standards of potability established by the American Dental Association. Regarding the origin of analyzed *S. aureus*, the most contaminated sites were high-speed handpieces in private offices (76%) and in dental care plan offices (71%), followed by air-water syringe in dental care plan offices (64%). For *S. epidermidis* samples, the most contaminated sites were high-speed handpieces in SUS (Brazilian Government Health System) dental offices (22%) and in dental care plan offices (14%). The most contaminated sites were dental offices that saw patients under dental care plans. Concerning tested antibiotics, the ones that presented better results as to sensibility to strain *S. epidermidis* were vancomycin and ciprofloxacin (100%) and, as to sensibility to strain *S. aureus*, it was ciprofloxacin (97%).

Key Words:
Staphylococcus spp, cross contamination, biofilm, dental office, water line

Introduction

In Dentistry, the control of cross infection is advised with the objective of reducing or eliminating the exposure of patients and dental staff to microorganisms, thus avoiding disease transmission. Therefore, a number of international entities, which regulate the dental practice, have launched standards for infection control, introducing measures for risk reduction and assurance of safer dental treatments. On the other hand, an important issue in the prevention of contamination at the dental office has been neglected: the quality of water used in the treatment[1].

Mills[2] says that the water line of dental equipment presents itself as an ideal means for microbial colonization and proliferation due to the extensive surface and a mild flow within the pipes. As a consequence of the bacterial biofilm, which is formed inside the pipes, the water deriving from the air-water syringe and the high-speed handpiece might have a high concentration of microorganisms.

The list of microorganisms, which have already been identified in the water supply of dental equipment, is long and variable[1]: *Bacillus subtilis*, *Enterecoccus ssp*, *Lactobacillus ssp*, *Pseudomonas aeruginosas*. Nevertheless, the literature is scarce regarding the isolation of *Staphylococcus spp*, a microorganism which is usually found in the clinical dental environment[2].

For Barbeau[3], *Staphylococcus*, which usually takes part in the regular microbiota of the host, does not take part in the biofilm of such water line; however, it might become opportunistic pathogens before a systemic unbalanced scene; therefore, there is a 10-fold increase of pathogenic bacteria over the surface and dental biofilm in individuals presenting poor dental health. Such fact might favor the introduction of bacteria in injured oral tissues as well as increase the reflux of such microorganisms for the water line pipe, which, whenever set by the air-water syringe and the high-speed handpiece, contaminate the surface of the dental equipment, predisposing to cross contamination risk among patients and staff by aerosol formation[4].

Considering the possibility of forming biofilm in water pipes, which contain bacteria populations deriving from a number of sources, such as water tanks with no maintenance, contaminated reservoirs, and the reflux of water from patients themselves during the surgical act, it is important to think about the control of the quality of water in dental equipment, reducing the risk of cross infection.

Therefore, the objective of the present study was to isolate and identify strains of *S. aureus* and *S. epidermidis* in water from dental offices in the city of Barretos-SP and to evaluate the sensibility of bacteria as to different antibiotics.

Material and Methods

During the period from January to July, 2005, there have been analyzed 160 samples of water from 40 dental offices strategically located in every neighborhood in the city of Barretos, São Paulo, in a way that such sampling represented the whole city. At these dental offices, water was collected from four different sites (high-speed handpiece, taps used for hand washing, water reservoir, and air-water syringe), and such equipment was not subjected to previous disinfection and collection was carried out during the afternoon, after four hours of equipment usage. In every dental office chosen for the research, the following items have been analyzed: kind of water reservoir (on the floor or in PET plastic bottles), water source (filtered, city supply system, or distilled), and equipment usage time.

Out of the total of collected samples, 14 derived from dental care plan offices, 9 came from SUS (Brazilian Government Health System) dental offices, and 17 came from private dental offices. Regarding 14 dental care plan offices, eight used filtered water and six used tap water from the city supply system, eleven used 500-mL PET bottles and three used reservoirs on the floor. As to 9 SUS dental offices, they all used tap water from the city supply system and individual 2000-mL reservoirs on the floor. All 17 private dental offices used filtered water and 500-mL PET bottles as reservoir. Two-hundred-mL was collected from each of the 160 samples, deriving from 40 dental offices with four samples each. Such samples were stored in previously sterilized 250-mL collection bottles with screw cap in a total of 160 collection bottles. Water from reservoirs on the floor was collected using 200-mL sterile disposable syringes. Water from high-speed handpieces was collected setting the pedal and pouring the flow in the collection bottle; the first flow was set apart for 30 seconds. Water from air-water syringes was directly drained inside the collection bottle. Samples of tap water were directly collected in the bottle. To every water sample, it was added 0.5-mL sodium thiosulphate in the final concentration of 10mg/L[5]. Samples were stored in thermal boxes with ice and transported to the microbiology lab of the Veterinary Pathology Department at FCAVJ-UNESP.

Water samples were filtered in 0.55-µm Millipore® filters, in a total of 200mL[5]. Following, every filter was placed in the center of Petri dishes containing *Staphylococcus* agar medium 110, which were incubated in a sterilizer at the temperature of 37ºC for 24-48 hours. Colonies with suspicion of belonging to gender *Staphylococcus* of white or yellow color and with shiny colonies formed over the culture medium were stained by the Gram method for observation of their morphotinctorial characteristics. Three colonies were picked from each sample and only colonies presenting positive morphotinctorial characteristics for the *Staphylococcus* gender were subjected to biochemical identification tests by means of evidences such as coagulase, catalase, hemolysis, mannitol fermentation, and Dnase.

Positive reference samples used in this work were: *S. aureus* ATCC 6538 and *S. epidermidis* ATCC 12228 (Fundação André Tosello – Campinas – SP).

Water quantitative bacteriologic analysis in cfu/mL were carried out using 0.55-µm Millipore® filter and they followed recommendations by the Standard Methods for the Examination of Water and Wastewater[5].

Each isolated one was tested by the disc diffusion method, according to recommendations by NCCLS[6] as to the susceptibility of the following antibiotics: ampicillin (10µg), amoxicillin (20µg), amoxicillin + clavulanic acid (10µg), azitromicin (15µg), cefazolin (30?µg), clindamycin (2µg), cloranphenicol (30µg), ciprofloxacin (5µg), novobiocin (10µg), oxacillin (6µg), and vancomycin (30µg).

Every result was subjected to the Fisher's exact test, at levels of 1 and 5% of probability in order to determine if differences were significant[7].

Results

From January to July, 2005, 160 samples of water from dental offices were studied. Out of the total of analyzed samples, 91.0 (57.0%) strains of *Staphylococcus spp* were isolated. From these, 77.0 (85.0%) were positive for *S. aureus* and 14.0 (15.0%) were positive for *S. epidermidis*.

From 40 studied dental offices, 15.0 (38.0%) used reservoir on the floor and 25.0 (62.0%) used plastic PET bottles. Among the reservoirs, 19.0 (47.0%) were supplied with tap water, 17.0 (43.0%) with filtered water, and 4.0 (10.0%) with distilled water. There was no statistically significant difference among samples which had been collected from reservoirs on the floor and plastic PET bottles, seeing that the contamination on these sites does not depend on the kind of water used (tap, filtered, or distilled) (table 1).

Regarding the period of usage for dental offices, eight (20.0%) had been used for less than five years and 32.0 (80%) had been used for more than five years. Therefore, we might say that, according to Fisher's Exact Test, equipment contamination by water does not depend on the period of usage of the dental office.

As to the origin of 77 analyzed strains of *S. aureus*, there have been isolated 13.0 (76.0%) samples from high-speed handpiece of private offices, 10.0 (71.0%) samples from high-speed handpiece of dental care plan offices, and 9.0 (64%) samples from air-water syringe (table 1).

As to the origin of 14 analyzed strains of *S. epidermidis*, there have been isolated 2.0 (22.0%) from air-water syringe of SUS dental offices, 2.0 (14.0%) from air-water syringe of dental care plan offices, and 2.0 (12.0%) from tap water of private offices. The most contaminated sites were dental care plan offices, followed by SUS dental offices and private dental offices (table 1).

Table 2 presents count results for *Staphylococcus spp* in cfu/mL of water samples that had been compared to the maximum standard of 200 cfu/mL recommended by the American Dental Association (ADA). Out of the total of

Table 1 - Number and percentage of samples of water contaminated by *Staphylococcus aureus and epidermidis* collected from different sites at 40 dental offices in Barretos-SP, during the period from January to July, 2005.

Collection sites	Contaminated samples			
	Staphylococcus aureus		*Staphylococcus epidermidis*	
	Number	%	Number	%
High-speed handpiece*:				
Dental care plans	10/14	71	2/14	14
SUS	5/9	55	2/9	22
Private	13/17	76	0/17	0
Tap:				
Dental care plans	7/14	50	0/14	0
SUS	4/9	44	0/9	0
Private	6/17	35	2/17	12
Reservoir:				
Dental care plans	6/14	43	0/14	0
SUS	3/9	33	1/9	11
Private	5/17	29	1/17	6
Air-water syringe*:				
Dental care plans	9/14	64	1/14	7
SUS	5/9	55	0/9	0
Private	8/17	47	1/17	6

* *Significant according to Fisher's Exact Test, p < 0.01*

Table 2 - Number of samples of contaminated water with strains of *Staphylococcus spp,* according to levels of contamination, in cfu/mL, in 40 dental offices in Barretos-SP, during the period from January to July, 2005.

Collection sites	Number of water samples	
	0 – 200 cfu/mL	201 – 2000 cfu/mL
High-speed handpiece	22	10
Tap	13	6
Reservoir	12	4
Air-water syringe	18	6

Table 3 - Profile of sensibility and resistance of strains of *Staphylococcus aureus* and *Staphylococcus epidermidis,* isolated from 40 dental offices in the city of Barretos-SP, during the period from January to July, 2005, as to different antimicrobials.

	Staphylococcus aureus					*Staphylococcus epidermidis*				
Antimicrobial	S	%	R	%	Total	S	%	R	%	Total
Ampicillin	28	36	49	64	77	7	50	7	50	14
Amoxicillin	33	43	44	57	77	4	29	10	71	14
Cloranphenicol	57	74	20	26	77	10	71	4	29	14
Novobiocin	46	60	31	40	77	8	57	6	43	14
Oxacillin	17	22	60	78	77	3	21	11	79	14
Vancomycin	70	91	7	9	77	14	100	0	0	14
Clindamycin	17	22	60	78	77	4	29	10	71	14
Azitromicin	65	84	12	14	77	8	57	6	43	14
Ciprofloxacin	75	97	2	3	77	14	100	0	0	14
Cefazolin	63	82	14	18	77	10	71	4	29	14
Amoxil + Clavulanic Acid	71	92	6	8	77	10	71	4	29	14

S = Sensibility; R = Resistance; % = Percentage of sensibility or resistance

analyzed samples, 65.0 (72.0%) were within standards recommended by ADA and 26.0 (28.0%) did not meet such potability standards.

Concerning the antibiotic sensibility and resistance profile, it can be observed that 97.0% samples of *S. aureus* were sensible to ciprofloxacin, 92.0% to amoxil + clavulanic acid, 91% to vancomycin, and 78% of samples were resistant to oxacillin and clindamycin (table 3).

For the studied profile of *S. epidermidis*, the antibiotics vancomycin and ciprofloxacin presented the best efficiency (100%), seeing that oxacillin (79.0%) and clindamycin (71.0%) were the ones that presented worse efficiency.

Discussion

According to the literature, water supplying the equipment is one source for cross contamination inside the dental office as it might work as a means of dissemination/transmission of microorganisms[8].

ADA[9] has established as a goal for surgeon-dentists a maximum bacterial load of 200 cfu/mL in water from air-water syringes and high-speed handpieces[10].

In this research, out of 91 samples of positive analyzed water for *Staphylococcus spp*, 65.0 were within potability standards recommended by ADA and 26.0 did not meet such standards, being able to be considered potential source of post-surgical and cross infection, seeing that the most contaminated site was high-speed handpiece, followed by air-water syringe, tap, and reservoir, these data are similar to Aguiar and Pinheiro[11] and Kohno et al[12].

According to Prevost et al.[13], the quality of water used for supplying the dental equipment cannot work as an indication of quality for affluent water since the water used for supplying the equipment presented an average of 15 cfu/mL whereas the effluent water was from $5.6x10^4$ to $9.0x10^6$ cfu/mL. These data are also confirmed in this work, as most samples of contaminated water from tap and reservoir do not exceed 200 cfu /mL.

In the present study, reservoirs on the floor as well as plastic PET bottles did not present differences as to water contamination, even being filtered water. This fact makes us believe that biofilm, which is formed in the inner wall of the water line pipe, is the cause of contamination of air-water syringes (21.0%) and high-speed handpieces (35.0%), seeing that it was used drinkable water, confirming then the results by Willians et al.[14]. It was still observed that the contamination of water in dental equipment does not depend on the period of usage of such equipment, which is in agreement with what has been stated by Watanabe[8].

In this study, sites where water has presented the highest levels of contamination were dental care plan offices, followed by SUS dental offices and private dental offices. Such fact might be a consequence of the fact that most dental care plans are medical-dental ones and patients are the same ones that go to the hospital environment[12].

Many surveillance studies have shown an increase in the prevalence of *S. aureus* resistant to oxacillin (SARO)[15]. SARO is recognized as one of the main causes of nosocomial outbreak, although with a great variation in several hospitals all over the country, in a rate from 26.6 to 71.0%[16]. The lethality assigned to infections caused by SARO is approximately 4.5 to 50%[15].

The oxacillin susceptibility tests have presented results similar to the ones found in the literature, 78% of isolated strains were resistant to such antimicrobial, with a little higher prevalence than the ones verified in the literature, which present 50 to 70% of the isolated ones resistant[17], national data report 66 to 68 % of isolated ones resistant to oxacilina[16], and national multicentrical data inform a total of 87% of resistance to *S. epidermidis*.

Regarding such facts, it might be concluded that the source of water contamination for air-water syringes and high-speed handpieces might be the reflux of water contaminated with *S. aureus* and *S. epidermidis* for hoses/water lines[14].

Concerning studied dental offices, dental care plan offices presented the highest level of contamination of water that comes in and out of the dental equipment.

It is also believed that patients with dental care plans have a higher level of appointments as they usually follow a treatment sequence, whereas patients from SUS and private dental offices go to the outpatient ward to solve emergency problems, which makes the dental care plan patient more likely to have contamination.

Strains *S. aureus* were sensible to the following antibiotics: ciprofloxacin, amoxil + clavulanic acid, and vancomycin, whereas strains *S. epidermidis* were sensible to ciprofloxacin and vancomycin, these data are similar to Miragaia et al.[16] and Chang et al[17].

In conclusion, it is worth suggesting that entities responsible for the dentistry practice, such as the Federal Board of Dentistry (CFO) and the Regional Board of Dentistry (CRO), as well as Schools of Dentistry should adopt measures for controlling cross infection within dental offices in order to assure better safety conditions for dentists, assistants, and patients through specialized training and valorization of life quality.

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