DIPARTIMENTO DI SANITÀ
PUBBLICA E MALATTIE INFETTIVE

SAPIENZA
UNIVERSITÀ DI ROMA

OGGETTO: Certificazione Sistema Bioster (Cefla Dental Group, Imola)

INTRODUCTION

Microbial biofilm formation in dental unit waterlines is documented by overwhelming evidence (Depaola et al., 2002; O'Donnell et al., 2011) and microbial levels in water samples of 10^4-10^5 colony forming units (cfu)/L are frequently reported. These micro-organisms are generally environmental, nonpathogenic species. However, opportunistic pathogens, such as *Pseudomonas aeruginosa*, *Legionella pneumophila* serogroup 1, non-tubercular *Mycobacterium*, are frequently detectable (Walker et al., 2004) and some cases of infection directly associated with contaminated dental unit waterlines are reported (Pankhurst and Coulter, 2007), including one lethal case of legionnaire's disease occurred in Italy where the contamination level of *L. pneumonia* serogroup 1 in dental unit waterlines as high as $6x10^4$ cfu/L was found (Ricci et al., 2012).

Micro-organisms come also from patients undergoing dental treatment and may gain access into dental unit waterlines in several ways, such as aspiration of biological fluids during the transient negative pressure that occurs when the dental turbine hand-piece stops rotating. Indeed, in more than two thirds dental units, oral streptococci, markers of salivary contamination, are detectable. Although dental unit waterline contamination level by oral streptococci is generally low (Petti et al., 2013), the fact that blood, saliva and other secretions from patients, who could be

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carriers of infections such as HBV, HCV and HIV cannot be ignored. In addition, opportunistic pathogens, such as members of the *Candida*, *Staphylococcus* and *Enterococcus* genera can be spread to immunologically compromised patients during dental therapy (Petti and Tarsitani, 2006; Szymańska and Sitkowska, 2013).

In order to overcome the problem of cross-infections transmitted through dental unit waterlines, several techniques have been proposed, such as water flushing before patient treatment and disinfection and, among disinfection methods, several products and systems are available (O'Donnell et al., 2011; Walker and Marsh, 2007).

The present is a technical study of the disinfectant activity of the Bioster system, (Cefla Dental Group, Imola, Italy) on micro-organisms from genera that are generally used to test the effectiveness of disinfectants and are also detectable into dental unit waterlines.

MATERIALS AND METHODS

Tested micro-organisms and cultivation

The tested micro-organisms were the following

- *Staphylococcus aureus*
- *Enterococcus faecalis*
- *Pseudomonas aeruginosa*

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- *Mycobacterium chelonae*
- *Candida albicans*
- *Legionella pneumophila* serogroup 1
- Spores of *Bacillus clausii*

As for *S. aureus*, *E. faecalis*, *P. aeruginosa*, *C. albicans*, swabs were collected from patients hospitalized at Policlinico Umberto I (Rome) and affected by Healthcare Associated Infections, due to micro-organisms that are generally resistant to biocides and antibiotics and are persistent in the environment (Calfee, 2012). Swabs were placed into saline solution (9 g/L NaCl), transported at 4 C temperature to the Department of Public Health and Infectious Diseases of the University of Rome "La Sapienza". The collected material was then plated on the following selective media:

- Mannitol Salt Agar (MSA –Becton Dickinson Italy, Milan, Italy) for the isolation of *S. aureus* and incubated at 37 C for 48 h in aerobiosis;
- Enterococcosel Agar (Becton Dickinson) for the isolation of *E. faecalis* and incubated at 37 C for 48 h in aerobiosis;
- Pseudosel Agar (Becton Dickinson) for the isolation of *P. aeruginosa* and incubated at 37 C for 48 h in aerobiosis;
- Bismuth Sulphite Glucose Glycine Yeast (BIGGY –Becton Dickinson) Agar for the isolation of *C. albicans* and incubated at 30 C for 48 h in aerobiosis.

After incubation, strains with typical colonies were Gram stained and identified through biochemical and antibiotic susceptibility tests using VITEK 2 (bioMerieux Italy, Florence, Italy).

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L. pneumophila was isolated from tap water of an old building, where it was found to be resistant to heat shock and hyperchlorination. 1 L of the sampled water was filtered with Millipore nitrocellulose membrane filters (pore sizes, 0.22 µm. Sigma-Aldrich Italy, Milan, Italy), resuspended in 10 mL of the original sample, vortexed for 30 sec, treated at 50 C for 30 min, diluted, plated on Charcoal-Yeast Extract (CYE –Becton Dickinson) Agar supplemented with Legionella BCYE-α Growth Supplement and incubated 10 days at 37 C in a 2.5% CO_2 atmosphere. Colonies with typical morphology were subcultured on CYE and BCYE and only colonies not grown on CYE were considered for the identification by means of agglutination tests using specific sera (Biolife Italy, Milan, Italy).

M. chelonae was isolated from tap water of a school where it was responsible for cutaneous infections. Sampled water was decontaminated with cetylpyridinium chloride (0.04%) and filtered with Millipore membranes (pore size, 0.45 µm, Sigma-Aldrich), plated on to Middlebrook 7H10 (Becton Dickinson), incubated at 37 C up to 6-10 weeks. Typical colonies were subcultured on BBL™ Lowenstein-Jensen Medium (Becton Dickinson) and incubated up to 6-10 weeks at 37 C. Developed colonies were Ziehl-Neelsen stained to detect acid-fast micro-organisms (D'Ancona et al., 2014). Then, Polymerase Chain Reaction to confirm mycobacteria to genera level and restriction analysis to identify to species level were performed (Briancesco et al., 2010).

Spore suspensions of *B. clausii* were bought (Enterogermina®; Sanofi-Synthelabo OTC, Milan, Italy).

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Procedure

Before any testing occasion, micro-organisms, excluding spores, were sub-cultured using the appropriate media and conditions. After incubation colonies were collected with a spatula and suspended in tubes containing 10 mL Hard Water (19.84 g $MgCl_2$, 46.24 g $CaCl_2$ in 1 L distilled water sterilized at 121 C for 15 min in autoclave) –HW.

For every strain and at every testing occasion the bacterial suspension in HW was equally distributed into two sterile tubes.

One sterile metallic net (**Figure 1**) was introduced in every tube for 1 h. During this period the nets were contaminated by the micro-organisms of the bacterial suspension. The tubes were stored into a bag at 4 C and transported to a dental office.

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Figure 1

The metallic net to be contaminated by the bacterial suspension

One net (Test net) was introduced into the waterline of a turbine-like instrument appositely designed to simulate massive contamination of the dental unit waterlines (**Figure 2**). Such an instrument was connected on a dental unit waterline system equipped with Bioster, a disinfecting system which operates cycles of waterline disinfection, by connecting this instrument in place of a turbine at the farthest point from the supply of the disinfectant. The cycle was the following, Namely, air flow to discharge water from the waterlines, flow of disinfectant (PeroxyAg+ --Cefla Dental Group, containing H_2O_2 3% v/v and Ag^+

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0.001% w/v), 10-min contact for disinfection, air flow to discharge the disinfectant, water rinsing to remove the residual disinfectant from the waterline. At the end of the cycle the net was aseptically removed from the instrument and put into a tube containing 5 mL HW and sodium thiosulfate (5 g/L), which was used to neutralize the residuals of the disinfectant. This neutralizer was chosen because effective against both H_2O_2 and Ag^+ and because of the lack of any antimicrobial activity (Kemp and Schneidert, 2000). The other net (Control net) was introduced into another turbine-like instrument filled with sterile HW, where it remained all the time the Test net underwent the Bioster cycle, and was then aseptically transferred into a tube containing 5 mL HW and sodium thiosulfate.

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Figure 2

The turbine connector to the dental unit waterline, the net and the turbine-like instrument designed to simulate a massive contamination of the dental unit waterlines (a). The contaminated net is inserted into the turbine-like instrument (b), which is connected to the turbine connector (c) and attached to the dental unit water system in place of the dental turbine at the farthest point of the waterlines from the supply of the disinfectant

a

b

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c

The tubes were stored at 4 C and transported to the laboratory of the Dental Section of the Department of Public Health and Infectious Diseases, where they were processed within 30 min.

The tubes were vortexed for 5 min, then 1:10, 1:100, 1:1000, 1:10000, 1:100000 dilutions were made into tubes containing HW. 0.5 mL of the undiluted suspension and of each dilution were plated in duplicate into plates containing the appropriate aforementioned media and incubated as previously explained. The remaining 4.5 mL of undiluted suspension were filtered with Millipore nitrocellulose membrane filters (pore sizes, 0.45 µm, Sigma-Aldrich Italy), the membranes were then plated and incubated. For every strain, the selective medium was used, while *B. clausii* was plated on to Tryptone Soy Agar (TSA –Becton Dickinson).

After the incubation period, colonies were counted and the bacterial loads were assessed for the Test and the Control nets and expressed as cfu. The use of specific selective media guaranteed that only the tested micro-organisms were counted and that micro-organisms potentially present in water were excluded. As

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for *B. clausii*, such a problem was overcome by counting only colonies with typical morphology.

For each strain the test was repeated five times.

Statistical analysis

For every strain, the bacterial loads detected with the Control nets were considered as markers of the level of contamination of the dental unit waterlines before the Bioster cycle, while loads detected with the Test nets were considered as markers of the residual level of contamination after the cycle. Therefore, in the present text contamination levels were indicated as Control or Pre-disinfection and Test or Post-disinfection, indifferently.

For every strain and every testing occasion, the Relative Reduction attributable to the Bioster cycle was assessed with the formula,

Relative Reduction = [(Control load) – (Test load)]/(Control load) x 100

For every strain, the mean Relative Reduction was assessed with 95% confidence interval.

Bacterial loads were log transformed to normalize the variances. Zero cfu was artificially treated as 1 cfu thus obtaining 0 log cfu when micro-organisms were undetected. The differences between the Control log loads and the Test log loads were assessed in order to estimate the number of log load reductions attributable to the Bioster cycle. The mean log loads also were assessed for every strain.

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RESULTS

The unit was connected to municipal water and was not used for patient treatment before the testing occasions. A disinfection cycle was performed the days before each testing occasions. Before the study start and 24 hours after the disinfection cycle, the contamination level of the dental unit water was tested for heterotrophic bacterial counts plating water samples and 1:10 dilutions on BD Bacto Yeast Extract (Becton Dickinson). One set of plates was incubated at 22 C and another set at 36 C for three days following previously described procedures (Castiglia et al., 2008). The resulting total viable counts were 42 cfu/mL and 49 cfu/mL at 22 C and 36 C, respectively, low enough not to interfere with the testing procedures. The physical characteristics of the outlet water from dental unit waterlines were rather stable. Namely, temperature ranged between 18 and 22 C, residual chlorine ranged between 0.08 and 0.25 mg/L, while pH ranged between 6.7 and 7.2.

Overall, excluding a long series of preliminary and pilot tests, 35 Tests and 35 Controls were performed.

The highest pre-cycle contamination levels were provided by *M. chelonae*, *S. aureus*, *E. faecalis*, *P. aeruginosa*, while the lowest levels were provided by *C. albicans* and *L. pneumophila* (**Table 1**). Post-cycle levels resulted null in all occasions with *S. aureus*, *E. faecalis*, *P. aeruginosa* and *C. albicans*, while there were always

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some micro-organisms remaining with *M. chelonae* and *B. clausii* spores and occasionally with *L. pneumophila*.

Table 1

Lowest and highest pre- and post-cycle contamination levels of the dental unit waterline (cfu) and frequency of occasions when the cycle resulted in complete elimination of micro-organisms

Micro-organism	Pre-cycle		Post-cycle		Percent
	Lowest level	Highest level	Lowest level	Highest level	Negative
S. aureus	11,100,000	20,100,000	0	0	100%
E. faecalis	9,300,000	24,300,000	0	0	100%
P. aeruginosa	7,800,000	21,900,000	0	0	100%
C. albicans	564,000	1,470,000	0	0	100%
L. pneumophila	705,000	948,000	0	90	60%
M. chelonae	3,000,000	96,000,000	32	465	0%
B. clausii (spores)	1,380,000	4,320,000	660	32,100	0%

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The mean Relative Reductions of dental unit waterline contamination levels attributable to the Bioster cycle, displayed in **Table 2**, could be broadly split into three groups. Namely, 100% with no variability (*S. aureus*, *E. faecalis*, *P. aeruginosa*, *C. albicans*), almost 100% with some variability (*L. pneumophila* and *M. chelonae*), less than 100% with high variability (spores of *B. clausii*).

Table 2

Mean Relative Reductions –and 95% confidence intervals- of dental unit waterline contamination levels attributable to the Bioster system

Micro-organism	Mean Relative Load Reduction	95% confidence interval
S. aureus	100.000%	100.000%
E. faecalis	100.000%	100.000%
P. aeruginosa	100.000%	100.000%
C. albicans	100.000%	100.000%
L. pneumophila	99.996%	99.992%-100.000%
M. chelonae	99.995%	99.989%-100.000%
B. clausii (spores)	99.632%	99.257%-100.000%

The highest mean pre-cycle log contamination levels were detected for *M. chelonae* *S. aureus*, *E. faecalis* and *P. aeruginosa* (higher than 7 log cfu), followed by *B. clausii* spores (6.5 log cfu) and *C: albicans* and *L. pneumophila* (close to 6 log cfu) (**Table 3**). Mean log reduction attributable to the Bioster cycle exceeded 7 logs for *S. aureus*,

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E. faecalis and *P. aeruginosa*, was almost 6 logs for *C. albicans*, more than 5 logs for *L. pneumophila* and *M.chelonae* and almost 3 logs for *B. clausii* spores.

Table 3

Mean log dental unit waterline contamination levels (log cfu), with 95% confidence intervals between parentheses, before and after the Bioster cycle with the various strains and mean log reduction attributable to the system

Micro-organism	Mean pre-cycle log level	Mean post-cycle log level	Mean log Reduction
S. aureus	7.160 (7.074-7.246)	0.000 (0.000)	7.160 (7.074-7.246)
E. faecalis	7.238 (7.087-7.389)	0.000 (0.000)	7.238 (7.087-7.389)
P. aeruginosa	7.174 (7.025-7.323)	0.000 (0.000)	7.174 (7.025-7.323)
C. albicans	5.982 (5.827-6.137)	0.000 (0.000)	5.982 (5.827-6.137)
L. pneumophila	5.920 (5.873-5.967)	0.746 (0.000-1.644)	5.174 (4.288-6.060)
M. chelonae	7.354 (6.678-8.030)	2.059 (1.602-2.516)	5.295 (4.172-6.418)
B. clausii (spores)	6.465 (6.296-6.634)	3.597 (2.815-4.379)	2.868 (2.180-3.556)

The effect of the Bioster cycle on micro-organisms that artificially contaminated the dental unit waterline is displayed in **Figures 3** and **4**. These figures together are helpful to show the strong decontaminating activity against *L. pneumophila* and *M. chelonae*, which resulted in more than 5-log load reduction –that is, after-cycle loads more than 100,000 times lower than post-cycle loads (**Figure 3**).

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Figure 3

Mean log dental unit waterline contamination levels (log cfu) assessed before (pre-cycle) and after (post-cycle) the Bioster cycle with the various strains

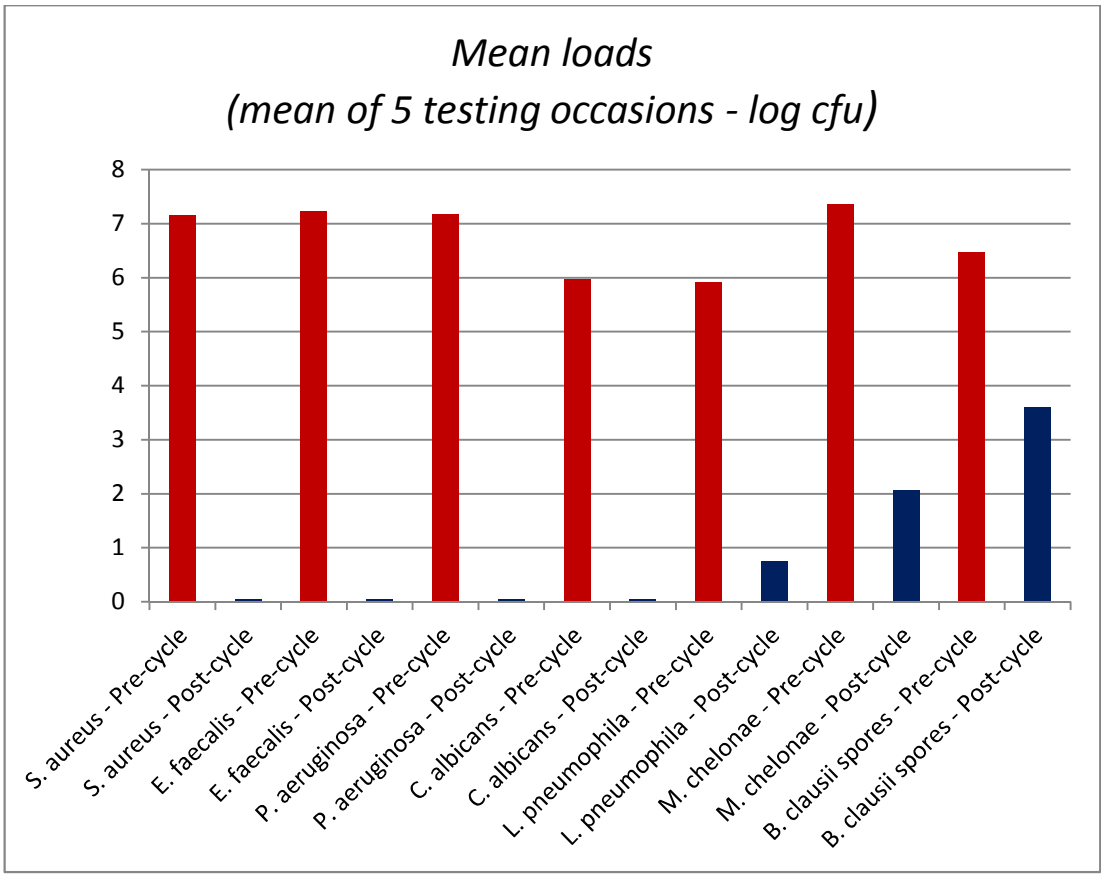

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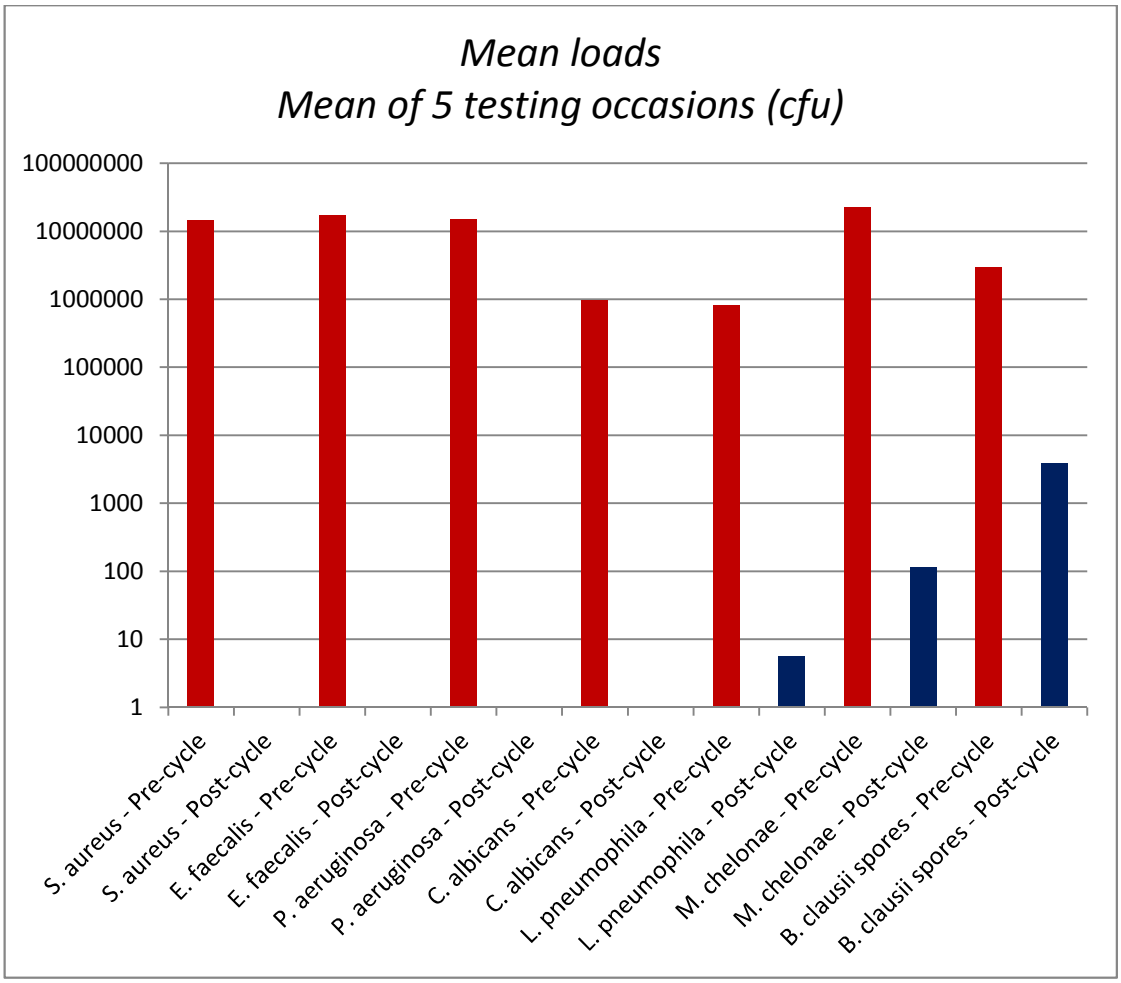
Figure 4

Mean dental unit waterline contamination levels (cfu) assessed before (pre-cycle) and after (post-cycle) the Bioster cycle with the various strains displayed on a logarithmic scale



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More specifically, *L. pneumophila* contamination levels of 1,000,000 cfu/mL were reduced to <10 cfu/mL after the disinfection cycle, while *M. chelonae* contamination levels higher than 10,000,000 cfu/mL were reduced to 100 cfu/mL after the disinfection cycle (**Figure 4**).

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DISCUSSION

Disinfectant activity

There are no EU standards to assess the effectiveness of disinfecting systems for dental unit waterlines like the Bioster system.

Thus, in order to evaluate the anti-microbial activity of this system, the reported data could be compared with the requirements provided by the European Committee for Standardization (CEN) to test the activity of disinfectants. It is important to note, however, that this and other disinfecting systems cannot be compared with disinfectants as the former are processes that are not exclusively based on the activity of the employed disinfectant. This important difference implies that a system cannot be tested in optimal laboratory conditions, but in real-life environment, namely, in the dental clinic with contact times, temperature, pH and other conditions that cannot range too much without wearing the instruments, altering the working conditions and the environment.

According to CEN, sporicidal activity (EN 14347:2005) is tested using spores of *Bacillus subtilis* subspecies *spizizenii* (American Type Culture Collection –ATCC-6633) and *Bacillus cereus* (ATCC 12826) at loads of 7 log cfu (basic limit –Nw, page 21 of the guideline). A sporicidal disinfectant is expected to decrease such a load by 4 logs within 30, 60, 90 min. In the present case, the use of spores of a different species and the log reduction by 2.9 logs (**Table 3**) suggest that the Bioster system is

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not comparable to a sporicidal disinfectant. Nevertheless, the fact that the cycle was able to inactivate more than 99% spores (**Table 2**) in only 10 minutes, suggests that the process has an intermediate sporicidal activity and, perhaps, a complete sporicidal activity if tested for a contact time of at least 30 min. Indeed, the sporicidal activity of 1% H_2O_2 is reported since 1940 with contact times longer than 5-6 hours (Curran et al., 1940).

According to CEN, mycobactericidal activity (EN 14348:2005) is tested using *Mycobacterium avium* (ATCC 15769) and *Mycobacterium terrae* (ATCC 15755) at initial loads of 9 log cfu (test suspension –N, page 12 of the guideline), while tuberculocidal activity is tested using *M. terrae* only. Disinfectants must show a reduction of at least 4 logs. Although in the present study only one mycobacterium was tested at a pre-cycle load of 7 log cfu (**Table 3**), the system was able to produce a decrease in contamination level by more than 5 logs, that is, more than ten times higher than what requested. Therefore, it is plausible that the antimicrobial activity of the Bioster system was comparable to a mycobactericidal and tuberculocidal disinfectant.

According to CEN, bactericidal activity against legionellae (EN 13623:2010) is tested using *L. pneumophila* serogroup 1 Philadelphia (ATCC 33152) at loads of 7 log cfu (basic limit $–N_0$, page 23 of the guideline). Disinfectants must show a reduction of at least 4 logs. Thus, although in the present study the pre-cycle load was only 6 log cfu (**Table 3**), not as high as in EN 13623:2010, the system was able to produce a decrease in contamination level by more than 5 logs and was comparable to an excellent anti-legionella disinfectant.

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Disinfectants yield fungicidal activity if they produce a 4-log decrease of *Aspergillus niger* (ATCC 16404) and *C. albicans* (ATCC 10231) at initial loads of 6 log cfu (basic limit $-N_0$, page 24 of the guideline) (EN 13624:2003), while yield yeasticidal activity if they are active against *C. albicans* only. Since *A. niger* was not tested it is not possible to say whether the system yielded fungicidal activity, however, with a 6-log decrease in *C. albicans* level (**Table 3**), the system is comparable to an excellent yeasticidal disinfectant.

Disinfectants are classified as bactericides if they produce a 5-log reduction of *S. aureus* (ATCC 6538), *Enterococcus hirae* (ATCC 10541) and *P. aeruginosa* (ATCC 15442) at loads of 7 log cfu (basic limit $-N_0$, page 21 of the guideline EN 13727:2003). Thus, although *E. faecalis* was used instead of *E. hirae*, the system, with a 7-log decrease (**Table 3**), is comparable to an excellent bactericidal disinfectant.

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In summary, the Bioster system was comparable with a disinfectant with complete

- yeasticidal activity,
- bactericidal activity,
- bactericidal activity against *L. pneumophila*,
- mycobactericidal and tuberculocidal activities

and intermediate

- sporicidal activity

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In addition to these excellent results, it is important to note that the criteria used in the present report to evaluate the anti-microbial activity of Bioster dental unit waterline disinfection system are more severe than those adopted by the Council on Scientific Affairs of the American Dental Association (ADA) to test dental unit waterline treatment devices. Indeed, the type of contamination used by ADA was a mixture of *P. aeruginosa* and *Klebsiella pneumoniae*, freshly isolated from the environment, at a standard concentration of 500 cfu/mL (American Dental Association Council on Scientific Affairs, 2014). Thus, the reported after-cycle *P. aeruginosa* contamination level of 0 cfu/mL obtained with the Bioster system (**Table 1**) would have led to ADA certification of effectiveness in the US.

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Practical effectiveness of the Bioster system in controlling cross-infections transmitted through Dental Unit Waterlines

Infection control guidelines in dental healthcare settings, including the most authoritative of them periodically released by the US Centers for Disease Control and Prevention, are –necessarily- only partly based on evidence. Indeed, when there are no data about infection risk and effectiveness of preventive measures, guidelines are based on the so-called Precautionary Principle, which states that "when an activity presents an uncertain potential for substantial harm to human health, precautionary measures should be taken even if there is no scientific evidence that such measures are needed or effective". According to this statement, however, implemented precaution-based preventive measures need to be periodically revised for effectiveness and toxicity and substituted by evidence-based measures. Therefore, precaution-based cross-infection control measures in dental healthcare settings need to be periodically tested whether they are effective in these specific settings (Petti and Polimeni, 2010).

Effectiveness and activity of dental unit waterline disinfection systems must, therefore, be tested and certified on the basis of their effectiveness against those micro-organisms and strains, that are specific cause of concern in dental healthcare settings, because of their spread in such an environment and because of their resistance to eradication both in the dental environment and among dental patients. According to this rationale, the effectiveness of the Bioster system was not tested theoretically using culture collection strains, that are susceptible to antibiotics and disinfectants or, at the best, their adaptive increased resistance to

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biocides is counterbalanced by lowered resistance to antibiotics and vice versa (Joynson et al., 2002). Instead, the system was practically tested using freshly isolated strains, responsible for acute diseases, resistant to several antibiotics and disinfectants, and widespread in the environment.

This rationale also led to test the effectiveness of the Bioster system against species that are different from those suggested by CEN, but are important pathogens in dental healthcare settings. For example, *E. faecalis* is detectable in dental unit waterlines (Petti and Tarsitani, 2006) and is responsible for 25-75% root canal treatment failures (Stuart et al., 2006). Therefore, it is likely that dental unit waterlines may spread these micro-organisms into the root canals during endodontic therapy thus causing endodontic infections. For this reason, *E. faecalis* was preferred to *E. hirae*, with no demonstrated pathogenicity. Again, *M. chelonae* is responsible for oral infections (Pedersen and Raible, 1989) and is detectable in dental unit waterlines (Schulze-Röbbecke et al., 1995), therefore, the antimicrobial activity against *M. chelona*e has more important consequences than the activity against *M. avium* and *M. terrae* that have never been detected in dental unit waterlines.

The present data were used to assess the effectiveness of the Bioster system to eradicate micro-organisms detectable in dental unit waterlines and at the highest reported level of contamination. The highest contamination levels of *S. aureus*, *E. faecalis*, *P. aeruginosa*, *C. albicans*, *L. pneumophila*, *M. chelonae* and *B. clausii* spores reported by literature in the last 25 years were searched using PubMed and Scopus as databases and “dental unit waterline” as key word. If quantitative data

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about one strain were not found, data regarding similar species were used. For every strain, the highest contamination level reported by the literature was compared with the log load reduction attributable to Bioster system. If the log load reduction was higher than the highest log contamination level, it was assumed that the Bioster system was effective in the eradication of that strain from the dental unit waterlines. The results are displayed in **Table 4**.

P. aeruginosa has been frequently detected in dental unit waterlines, the highest reported level was $2x10^5$ cfu/mL (Barbeau, 2000). The Bioster system decreased the *P. aeruginosa*-contamination level by 7 log cfu. Thus, disinfectant activity was high enough to eradicate these micro-organisms from dental unit waterlines.

Staphylococci also have been detected in dental unit waterlines, the highest reported contamination level was $1.5x10^4$ cfu/mL (Szymańska and Sitkowska, 2013). Once again, the disinfectant activity of the Bioster system was high enough to decrease the *S. aureus* contamination level to null.

Enterococci have been detected in dental unit waterlines (Petti and Tarsitani, 2006), the highest reported contamination level was $1.6x10^3$ cfu/mL (Szymańska and Sitkowska, 2013). On the basis of the present data, the disinfectant activity of the Bioster system was high enough to prevent the transmission of these micro-organisms through dental unit waterlines.

Candida micro-organisms have been seldom isolated from dental unit waterlines, the highest detectable level ever reported was $6.6x10^1$ cfu/mL

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(Kadaifciler et al., 2014), thus suggesting that Bioster is able to neutralize all *Candida* micro-organisms from dental unit waterlines.

Legionellae have been frequently (Walker et al., 2004) and persistently (Petti et al., 2004) detected in dental unit waterlines, the highest reported contamination level of *L. pneumophila* was $8x10^3$ cfu/mL (Ma'ayeh et al., 2014). Since the Bioster system resulted in a 5-log load decrease it is able to prevent the transmission of *L. pneumophila* serogroup 1 through dental unit waterlines.

Mycobacteria have been frequently detected in dental unit waterlines, the highest level of contamination was $2.1x10^3$ cfu/mL (Schulze-Röbbecke et al., 1995). The disinfectant activity of the Bioster system was high enough to prevent the transmission of these micro-organisms through dental unit waterlines.

Table 4 (displayed in the following page)

Anti-microbial activity of the Bioster system (log cfu reduction), compared with the highest dental unit waterline contamination levels (log cfu/mL) reported by literature. Consequent effectiveness in eradicating micro-organisms in clinical-like conditions (log cfu reduction minus highest log cfu)

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Micro--organism	Anti-microbial activity	Micro-organism	Contamination level	Effectiveness
S. aureus	7.160	*Staphylococcus* spp.	4.176	YES (<1 cfu remaining)
E. faecalis	7.238	*E. casseliflavus*	3.204	YES (<1 cfu remaining)
P. aeruginosa	7.174	*P. aeruginosa*	5.301	YES (<1 cfu remaining)
C. albicans	5.982	*C. formata*	1.820	YES (<1 cfu remaining)
L. pneumophila	5.174	*L. pneumophila*	3.919	YES (<1 cfu remaining)
M. chelonae	5.295	*M. chelonae* and *M. gordonae*	3.313	YES (<1 cfu remaining)
B. clausii spores	2.868	*B. halodurans**	3.059	YES (<1 cfu remaining)

*spores usually are <10% of the global load, therefore, it is plausible that spore load was 2.059

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Spore-forming bacteria –only a small fraction, close to 10%, are spores- have been detected in dental unit waterlines at a highest level of $1.2x10^3$ cfu/mL (Szymańska and Sitkowska, 2013). Since the Bioster system caused a spore decrease by 2.9 log loads, it is plausible that even spore transmission can be prevented.

In summary, the disinfectant activity of the Bioster system was enough to permit the eradication of all the tested micro-organisms from the dental unit waterlines, even at the highest contamination levels, thus resulting in a total protection against micro-organisms transmission in the dental healthcare settings.

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Rome, december 16th 2014

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